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CYTOLOGICAL STUDIES OF HELMINTHOSPORIUM SATIVUM[1]

BY S. B. HRUSHOVETZ[2]

Abstract

Cytological studies of *Helminthosporium sativum* P.K. & B. showed that a mechanism exists for the perpetuation of heterokaryons. Septa are laid down in such a way that usually the hyphal tips and mycelial cells are initially multinucleate. Hyphal fusions occur in all parts of the colony and nuclei have been observed in the bridging hyphae. Two or more nuclei generally enter the young conidiophore and several nuclei invariably enter the young conidium before the latter is delimited from the conidiophore. The multicellular, multinucleate conidia exhibit polar or bipolar germination but only the nuclei in the terminal cells of the conidium enter the germ tubes; the remaining cells and their nuclei subsequently degenerate. Germination of complete conidiophores or portions of conidiophores was also observed.

Introduction

Monoconidial and hyphal tip isolates of *Helminthosporium sativum* are known to yield numerous physiological (1) and morphological (2, 8, 15) variants, some of which consistently produce sectors. In a cytological study, Christensen and Davies (2) found that only rarely does more than one nucleus enter the young conidiophore and conidium. They concluded, therefore, that the occurrence of variants and new strains in this species is attributable mainly to new mutations, heterokaryosis assuming a minor role, if any. Dickinson (4) had earlier obtained similar findings with three species of *Helminthosporium*. The cultural studies by Shands and Dickson (14) of hyphal tips from monoconidial isolates and the cytological studies by Graham (5) suggest that in the related species *H. gramineum*, heterokaryosis may play an important role in variability. More recently Roane (11) showed by cytological and cultural studies of *H. carbonum* that in this fungus "heterokaryosis may be perpetuated indefinitely". Although Tinline (16) based his studies of genetics and pathogenicity of *H. sativum* on assumed homokaryosis of monoconidial isolates, he does not entirely exclude heterokaryosis as a possible cause of variation.

[1]*Manuscript received October 7, 1955.*
Joint contribution from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario,—Contribution No. 1496—and the Department of Botany, University of Toronto, Toronto, Ontario. The work in this paper is from a thesis presented to the Graduate School of the University of Toronto in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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[The March number of this Journal (Can. J. Botany, 34 : 209-320. 1956) was issued March 15, 1956.]

Consideration of the investigations cited above indicated that uncertainties exist concerning the origin and distribution of nuclei in the hyphal cells and conidia of *H. sativum*. Further work on these aspects seemed desirable as a basis for studies on the physiology and pathogenicity of this fungus.

Materials and Methods

Culture material used in the present study consisted of three strains of *H. sativum*: a subculture of the albino strain of Greaney and Machacek (6), and two wild type or normally pigmented strains which were monoascosporic isolates of a compatible mating pair from R. D. Tinline, Saskatoon. Preliminary studies of the wild type cultures showed that the brittleness and pigmentation of conidiophore and conidial wall (episporium of de Bary (15)) impeded stain penetration and visibility of the stained nuclei. Consequently cytological studies of these normal pigmented strains were mainly restricted to the mycelium and very young conidiophores.

A modification of the cellophane square method as used by Roane (12) for *H. carbonum* and Yuill (17) for *Aspergillus* was employed. The plain transparent grade (300PT) of cellophane secured from W. J. Bell Paper Co. Ltd., Toronto, was found most satisfactory. One-inch squares of cellophane were boiled for 15 min. in distilled water to sterilize them and to remove wax or grease, transferred aseptically to the surface of nutrient agar medium, and on the agar arranged in the form of a triangle or square (Fig. 1). Fungus inoculum was placed directly on the agar in the center of the plate. Sachs' agar medium (16) was used because it limited the production of aerial mycelium and greatly reduced the growth of contaminants. When the fungus mycelium reached the cellophane it grew on the upper surface rather than between the cellophane and agar. For study of germination, conidia were placed directly on the cellophane. When the desired stages had developed, the cellophane squares containing the fungus mycelium were carefully removed from the plates and placed in a Carnoy type of fixative (three parts absolute ethyl alcohol, one part glacial acetic acid) for 10 min. After fixation the cellophane was rinsed in 95% ethyl alcohol and then transferred to 70% ethyl alcohol where it could be stored for several days before staining.

The staining procedure was a modification of the HCl–Giemsa method used by Robinow (13). A report on cytological studies of the ascomycete *Cochliobolus sativus*, now being prepared by the author, shows that the Giemsa stain as used is specific for chromosomes. The fixed material was rinsed in water, immersed in cold *N* HCl for five minutes, hydrolyzed in *N* HCl at 60° C. for seven minutes, washed thoroughly in distilled water, rinsed in phosphate buffer (pH 6.9), and finally placed in the Giemsa staining solution for two hours. The phosphate buffer was prepared as follows: to 50 cc. of *M*/5 KH_2PO_4 enough *M*/5 NaOH was added to bring the pH up to 6.9; distilled water was then added to make 200 cc. of buffered solution. Ten drops of the Giemsa stain, as prepared by the British Drug Houses, were added to 5 cc. of phosphate buffer and this was used as the staining solution.

The cellophane squares were removed from the staining solution, rinsed in distilled water, and placed in a Petri plate containing the phosphate buffer. Here they were cut into smaller pieces and transferred directly to a microscope slide containing a drop of buffer solution. A cursory microscope examination determined whether further destaining in distilled water was required. Semipermanent mounts were made in a water soluble mounting medium consisting of the phosphate buffer saturated with Abopon. Abopon may be used without dilution but often large crystals form in the prepared mount or in the bottle containing it.

Observations and photographs were made with a Leitz Wetzlar Ortholux microscope fitted with a fluorite objective (42×, N.A. 0.85), periplan compensating eyepieces (6× and 10×), and a double diaphragm condenser (N.A. 1.40). The microscope contained a built-in illuminating system and was supplied with an amperage control and provided with a yellow-green Wratten filter. Photographs were taken with a Bausch and Lomb microscopic attachment on Kodak Plus X panchromatic film, developed in Microdol, and printed on Kodabromide F4 paper.

Results

Unless otherwise specified all observations refer to the pink mutant or albino strain of *H. sativum*.

The nuclei in the mycelium stained a deep reddish-purple while the cytoplasm remained colorless or stained a very faint blue. The nucleolus, which did not stain, was present as a colorless oval body within the nuclear mass. The number of nuclei in the vegetative cells of the mycelium varied from 0 to 13, with 2 to 5 the most common (Fig. 2). Hyphal fusion with subsequent nuclear migration through the bridging hyphae was observed near the hyphal tips (Fig. 3) or whenever two older hyphae lay parallel and close to each other. Older hyphae frequently formed 'mycelial ropes' (15). Migration of nuclei through septa or cross walls was not observed. Pseudofusions, that is hyphal fusions in which there is no cytoplasmic continuity (2) and therefore no possibility of nuclear migration, were also observed (Fig. 6). At the time that the septum is laid down in the terminal cell of a hypha, the hyphal tip so formed may be uninucleate, binucleate, or multinucleate. For example, in the last three cells of a hypha, the number of nuclei were as follows: ultimate cell five, penultimate cell three, third cell four. If the ultimate and penultimate cells were originally uninucleate it is not likely that the presence of five and three nuclei, respectively, in these cells is due to continued divisions of the original nuclei. This would necessitate radically different rates of division of the daughter nuclei following the first mitosis in each cell. There is no evidence that such a phenomenon exists in the hyphal tips of *H. sativum*. It is more probable that the hyphal tip contained eight nuclei and that a cross wall appeared cutting off five nuclei in the ultimate cell and three nuclei in the penultimate cell.

Conidiophores arose as lateral branches of the main hyphae. In the wild type they quickly became pigmented and could easily be distinguished from ordinary lateral hyphae (Fig. 4). Figs. 4, 5, and 6 show that at least two nuclei entered the young conidiophore of the dark-colored mating type (*A*) strain. However, sometimes cross walls were laid down so rapidly that the individual cells of the conidiophore were uninucleate. The entrance of only one nucleus into the conidiophore rarely occurred. In the mating type (*a*) strain the young conidiophores were less pigmented and almost invariably more than two nuclei were seen in them. Complete conidiophores or portions of conidiophores of the wild type strains often germinated and functioned as conidia (Fig. 7). In the albino strain the distinction between a lateral hypha and a young conidiophore could only be made after the conidiophore became septate (Fig. 8). It was therefore very difficult to determine in this strain the number of nuclei which entered the very young conidiophore.

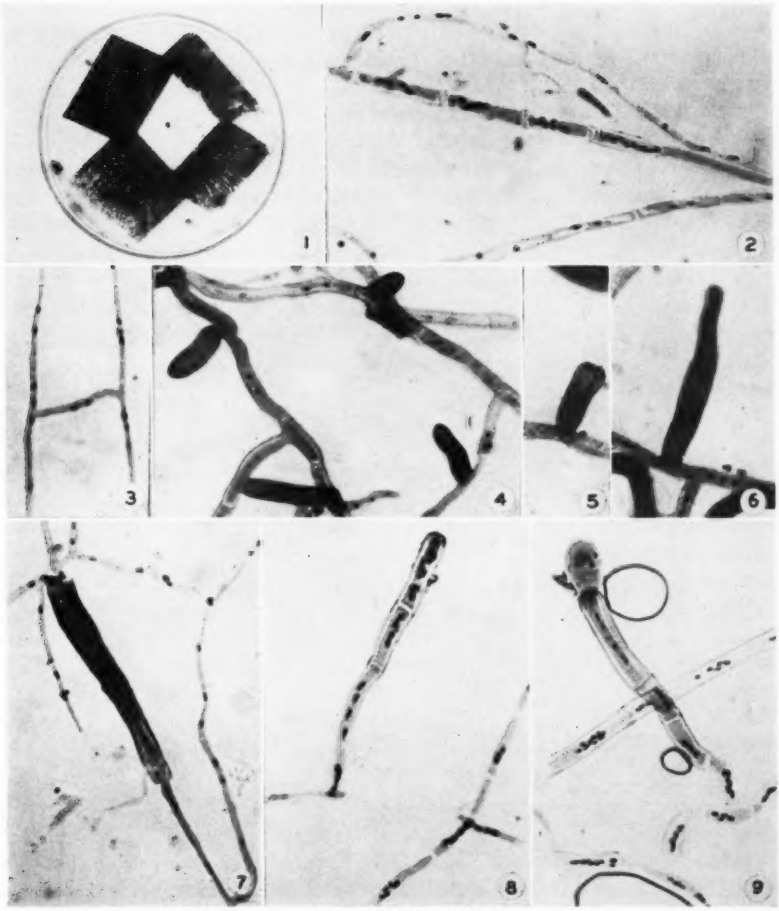
Conidia were produced on multinucleate, multicellular, unbranched conidiophores. The first conidium appeared as an apical outgrowth of the terminal cell of the conidiophore. As this swelling enlarged, several nuclei entered it and the young unicellular conidium was then cut off from the conidiophore (Fig. 9). Additional conidia arose from successive new growing points which developed laterally to the apex (Fig. 10). Various abnormalities were noted, viz., the first conidium may develop laterally to the apex, the primordium for the second conidium may arise from subterminal cells, or various primordia may themselves become new growing points before they produce conidia.

Although several nuclei invariably entered the primordium of the young conidium (Figs. 9, 10), numerous nuclei always remained in the terminal conidiophore cell (Fig. 11). As the conidium elongated cross walls were laid down very rapidly and the spore became multicellular. These walls were formed by ingrowths of the inner hyaline layer or endosporium of the conidium and the cells so formed were completely separate from each other. Progressive elongation of the conidia often involved a further subdivision of each cell; as many as 13 cells have been counted in some conidia. The cells resulting from these subdivisions were always multinucleate.

Conidia exhibit polar or bipolar germination. Only the terminal cells of a conidium normally function in germination (Figs. 12, 13). Several nuclei from these terminal cells invariably entered the young germ tube. The remaining cells of the conidium subsequently degenerated. Only occasionally did germ tubes develop from these interstitial cells, and these were always from abnormal conidia.

Discussion

It is apparent from the results of the present study that it is unnecessary to postulate an abnormally high mutation rate for *H. sativum* (*vide infra*), and that the existence of numerous variants reported by earlier workers (1, 2, 15), can, in principle, be interpreted in terms of heterokaryosis with occasional nuclear dissociation.



Unless specified all photographs are of the pink or albino mutant. (× 712). Fig. 1. Two-week-old culture of a normal dark strain of *Helminthosporium sativum* grown on Sachs' agar medium containing squares of cellophane. Note enhancement of conidiophore and conidium production by the cellophane. Fig. 2. Hyphal cells showing variability in numbers of nuclei. Fig. 3. Hyphal fusion near the hyphal tip. Two nuclei are present in the bridging hypha. Figs. 4–6. Young conidiophores of the dark strain T_2(A); where visible at least two nuclei are seen entering the young conidiophores. Fig. 6. A mature septate conidiophore. The short bridging hypha is actually a pseudofusion. Fig. 7. Germination of a portion of a conidiophore of the dark strain T_2(A). Note early hyphal fusions. Fig. 8. Young multinucleate, multicellular conidiophores. Note entrance of several nuclei into the basal cell. Fig. 9. Conidiophore producing first conidium. Several nuclei are present in the young conidium at the time it is delimited from the conidiophore.

PLATE II

All photographs are of the pink or albino mutant (× 700). Fig. 10. Entrance of several nuclei into the primordium of the second conidium. Fig. 11. Multinucleate, multicellular first and second conidia. Note the presence of numerous nuclei in the terminal cell of the conidiophore. Fig. 12. Polar germination of mature conidium. The large cell in the upper conidium contains over 30 nuclei. Fig. 13. Bipolar germination of a mature conidium. Only the nuclei in the terminal cells of the conidium enter the germ tubes.

When Christensen and Schneider (3) compared the monoconidial progenies of a monoconidial isolate grown continuously on potato-dextrose agar with that grown on a wheat plant, they found that "with the exception of five mutants, 14,400 monosporous (monoconidial) progenies from the host were identical in cultural characters with the original line. The frequency of mutation of *H. sativum* on the host was approximately 1 : 2900." On the other hand no mutants at all were found in isolates taken directly from potato–dextrose agar. The factor of differential selection of mutants by the wheat plant and potato–dextrose agar which these authors apparently did not consider, together with the present cytological findings of the multinucleate nature and origin of the vegetative structures of this fungus, strongly suggest that heterokaryosis with subsequent nuclear dissociation may account for part of this abnormally high mutation rate.

Hyphal fusions may produce and maintain heterokaryosis. The present study showed that in the pink strain anastomosis with actual nuclear migration through the bridging hyphae occurred in any part of the colony. Tinline (16) has shown cytologically that hyphal fusions can occur between the pink and dark strains of *H. sativum*. He also found that out of 712 hyphal tip isolates from cultures of *H. sativum* containing a mixture of a pink and a dark strain, 14 intermediate colonies were produced; the remainder gave colonies comparable to either parent. These intermediate colonies consisted predominately of hyphae of the dark strain but also possessed rings of the pink mycelia. Hyphal tip isolates from these colonies yielded intermediate types and the wild type parent, but not the albino parent. The present author believes that this demonstrates the persistent association in the hyphal tips of the two genetically different nuclei. Christensen and Davies (2), on the other hand, support the thesis that whenever two genetically different nuclei are found in the same cell, either as a result of mutation or hyphal fusion, the existence of the heterokaryon is only temporary and the two nuclei quickly dissociate.

The recent findings of Tinline (16), that in mixed cultures of a pink and a dark strain of *H. sativum* all the conidia found on the same conidiophore were of one color type only, appear to support the thesis that conidiophores of this fungus are homokaryotic. However, the occurrence on individual conidiophores of conidia of one color type only is not conclusive proof that the conidia or the conidiophores are actually homokaryotic. Pontecorvo (9) has shown that although in some fungi color of conidia may be autonomously controlled by the nucleus in the conidium, in others, e.g. *Penicillium notatum*, it may be governed by the nuclei of the conidiophore or even by the nuclei of the mycelium. It is possible that in *H. sativum* determination of pigmentation of conidia is of the latter type.

Cultural and cytological studies of species of the genus *Helminthosporium* (5, 7, 11, 14), as well as species of other Fungi Imperfecti (7, 10, 17), furnish evidence of a wide distribution of heterokaryosis. The present study of production, development, and germination of conidia also showed that a

cytological pathway exists for the perpetuation of heterokaryons in *H. sativum*. Several nuclei entered the young conidium before it was delimited from the conidiophore, and as the conidium elongated new cells arose in it by a cleavage of the existing cells. It is important to note that the cells resulting from fission were multinucleate from the beginning and that their nuclei continued to divide because mature spores were found to contain as many as 30 nuclei in a cell. As it was observed that only the nuclei of the terminal cells of the conidium entered the germ tubes, the perpetuation of a heterokaryon through this part of the asexual life cycle is determined in part by the distribution of nuclei during the early fissions and by the mode of conidium germination. If one assumed that two genetically different nuclei were present in the unicellular conidium and that they dissociated in the first fission, a heterokaryon could only be perpetuated by bipolar germination and early hyphal fusions between the germ tubes. If no anastomosis occurred, then such conidia will invariably produce 'half sectors', i.e., the heterokaryon will be permanently dissociated. If on the other hand, they did not dissociate at the first or later fissions, then polar germination alone could perpetuate the heterokaryon. It is obvious that if dissociation occurs in these early fissions in such a way that the two terminal cells are genetically alike, then a heterokaryon would not be perpetuated even by bipolar germination and early hyphal fusions between the two germ tubes from the terminal cells of the conidium.

Although this study has demonstrated that a cytological mechanism exists in *H. sativum* for the association of diverse kinds of nuclei in all vegetative structures, a heterokaryon once formed may not be perpetuated indefinitely. Besides the chance formation of homokaryons in multinucleate cells by the random nuclear dissociation of heterokaryons, the occasional occurrence of uninucleate vegetative structures in this fungus offers a mechanism for nuclear dissociation. From the studies of Pontecorvo (10), it is obvious that the production, persistence, and dissociation of heterokaryons can be studied in more detail if biochemical as well as additional morphological mutants are used.

Acknowledgments

The writer is deeply indebted to Dr. K. H. Rothfels, Department of Botany, University of Toronto, for his constant advice and suggestions throughout the course of these studies and in the preparation of the thesis and manuscript; and to Professor J. Morgan-Jones, University of Toronto, for critically reviewing the thesis. Thanks are especially due to Drs. J. E. Machacek and R. D. Tinline, Plant Pathology Laboratories, Science Service, at Winnipeg, Man., and Saskatoon, Sask., for supplying the strains of the fungus.

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CRESTED WHEATGRASS COMPLEX[1]

By P. Sarkar[2]

Abstract

In the complex of taxa commonly called crested wheatgrasses, at least five species can be distinguished according to descriptions by Nevski in the Flora of the U.S.S.R. These are: *Agropyron cristatum*, *A. pectiniforme*, *A. desertorum*, *A. michnoi*, and *A. sibiricum*. Of the two commercial forms of *A. cristatum*, 'Standard' and 'Fairway', the latter is diploid with $2n=14$. From a consideration of its chromosome number and morphological distinctness this taxon is here given specific status with the name *Agropyron cristatiforme*. Each of the five other species mentioned above is tetraploid with $2n=28$. Karyomorphologically all the species of *Agropyron* studied here are characterized by chromosomes with median or submedian centromeres and by the lack of any secondary constriction or satellite. A high frequency of multivalents in the pollen mother cells of *A. michnoi* and *A. sibiricum* indicates an autoploid derivation of these two species.

Introduction

The wheatgrasses comprise a great many species with complex interrelationship. The taxonomic disorder in this group of grasses is surprising, considering the fact that the component species are of interest from the practical as well as academic point of view, since some of them have already played an important role in the improvement of pasture grasses, and some are considered to be potential donors of many desirable characters to the cultivated wheat. As matters stand today, there is still so much confusion in nomenclature, even at the generic level, that it is sometimes impossible to ascertain what material is in use in reported studies. An attempt to establish a basis for taxonomic clarification of the group is urgently needed. For an appraisal of the present confusion and the recent attempts to reclassify this group of grasses, it seems desirable to trace the taxonomic history of these taxa.

Linnaeus (10) recognized eight species of wheatgrasses, six of which he placed in the section *Perennia* of the genus *Triticum*. Of the other two, he placed one in *Secale*, and the other in *Bromus*. Linnaeus observed the heterogeneity among the different species, since he separated *cristatum* and *orientale* from the other species and put them in *Bromus* and *Secale* respectively. It was Gaertner (5) who first placed *cristatum* in the new genus *Agropyron* together with the new species *triticeum*. Marschall von Bieberstein (13) tried to include *Secale orientale* in *Triticum*, but later it was also transferred to the genus *Agropyron* by Roemer and Schultes (1817).

Palisot de Beauvois (19) transferred all the Linnaean species of perennial *Triticum* to the genus *Agropyron*, in spite of the fact that two years earlier Desvaux described the new genus *Elytrigia* to include the species *repens*.

[1]*Manuscript received January 9, 1956.*
Contribution from Department of Botany, University of Manitoba, Winnipeg, Canada. Based on part of a thesis submitted in April, 1955, to the Faculty of Graduate Studies and Research of the University of Manitoba in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
[2]*Research Assistant, Department of Botany, University of Manitoba, Winnipeg, Canada.*

Ledebour (9), instead of separating the wheatgrasses from *Triticum*, placed *cristatum* and *triticeum* in the new section *Eremopyrum*. Later, Jaubert and Spach (7) gave *Eremopyrum* generic status and included in it the annual species *orientale* and *squarrosum*. The recognition of *Eremopyrum* as a separate genus was very fortunate, since it provided the basis for later grouping of the annual wheatgrasses which, apart from their annual habit, are karyomorphologically distinct from all other species of the *Triticinae* (22).

In recent years, the Russian taxonomist Nevski (16, 17, 18) attempted to reclassify the wheatgrasses and their relatives. Summarizing briefly (18) it may be noted that he divides the tribe *Hordeeae* into subtribes, three of which, *Roegneriinae*, *Aegilopinae*, and *Agropyrinae* include the five genera, *Roegneria*, *Anthosachne*, *Eremopyrum*, *Agropyron*, and *Elytrigia*, into which are incorporated all the species of *Agropyron* in its wider sense. *Roegneria* (including such species as *caninum* and *trachycaulum*, etc.) and *Anthosachne* are included in the subtribe *Roegneriinae*. *Eremopyrum*, together with *Triticum*, *Secale*, *Aegilops*, *Haynaldia*, and *Heteranthelium* is placed in the subtribe *Aegilopinae*. The rest of the wheatgrasses fall into the genera *Elytrigia* and *Agropyron* in the subtribe *Agropyrinae*. The genus *Elytrigia* contains such species as *repens*, *junceum*, *intermedium*, *trichophorum*, *elongatum*, *spicatum*, etc. while the genus *Agropyron* includes only the species with more or less dense and crested spikes.

Melderis (14) on the basis of his own morphological and genetical studies of the members of the tribe *Hordeeae*, as well as on the cytogenetical evidence found in the literature, proposed some modifications in Nevski's system. So far as the wheatgrasses are concerned, Melderis' modified system differed from Nevski's in that here *Eremopyrum*, instead of being grouped together with other annual genera, is placed together with *Elytrigia* and *Agropyron* in the subtribe *Agropyrinae*.

To seek justification of these recent attempts to reclassify the wheatgrasses as a whole is beyond the scope of the present investigation, and can hardly be attempted until more cytogenetic data have been accumulated. Here only crested species that fall under *Agropyron* (according to Nevski's classification) will be dealt with. These are considered to be the crested wheatgrasses.

Material and Methods

The seed samples of *A. cristatum* (L.) Gaertn. var. 'Fairway' were obtained from the following sources:

(1) Ottawa, Canada, C.A.N. 5074. P-3.

(2) Botanical Garden, Marburg, Germany, 106. P-40.

(3) Northern Great Plains Field Station, Mandan, North Dakota, U.S.A., D1411. P-43.

(4) Budapest, Hungary. P-53.

(5) Botanical Garden, Berlin – Dahlem, Germany, 152. P-55.

The rest of the species of *Agropyron* investigated here are listed as follows: *A. cristatum* (L.) Gaertn.; *A. pectiniforme* Roem. and Schult.; *A. desertorum* (Fisch.) Schult.; *A. michnoi* Roshev.; *A. sibiricum* (Willd.) P.B. These were obtained as seeds and living specimens from the Dominion Experimental Farm, Brandon, Manitoba, Canada.

In identifying *A. pectiniforme*, *A. desertorum*, and *A. michnoi*, photographs of type and cotype specimens from the V. L. Komarov Botanical Institute of the Academy of Sciences of the U.S.S.R. were used for comparison. *A. cristatum* was compared with herbarium specimens from the same institute. For the identification of *A. sibiricum*, photographs of specimens from the former herbarium of Willdenow, obtained through the Botanical Garden and Museum, Berlin – Dahlem, were used.

To determine somatic chromosome numbers and for the study of chromosome morphology, seeds were germinated in Petri dishes with moist filter paper, and root tips collected when the roots had grown to a length of about 0.5 to 1 cm. Temporary squash preparations were made according to Tjio and Levan's (28) oxyquinoline method with excellent results.

Meiotic preparations were made according to Belling's iron–acetocarmine squash method after the spikes had been fixed in a modified Carnoy's solution (absolute alcohol: chloroform: glacial acetic acid: : 6 : 3 : 1) at least overnight. Good preparations could be made even after the spikes had been kept in Carnoy's solution for indefinite periods of time. The slides were made permanent by detaching the cover glass in a jar of 95% alcohol and mounting in euparal.

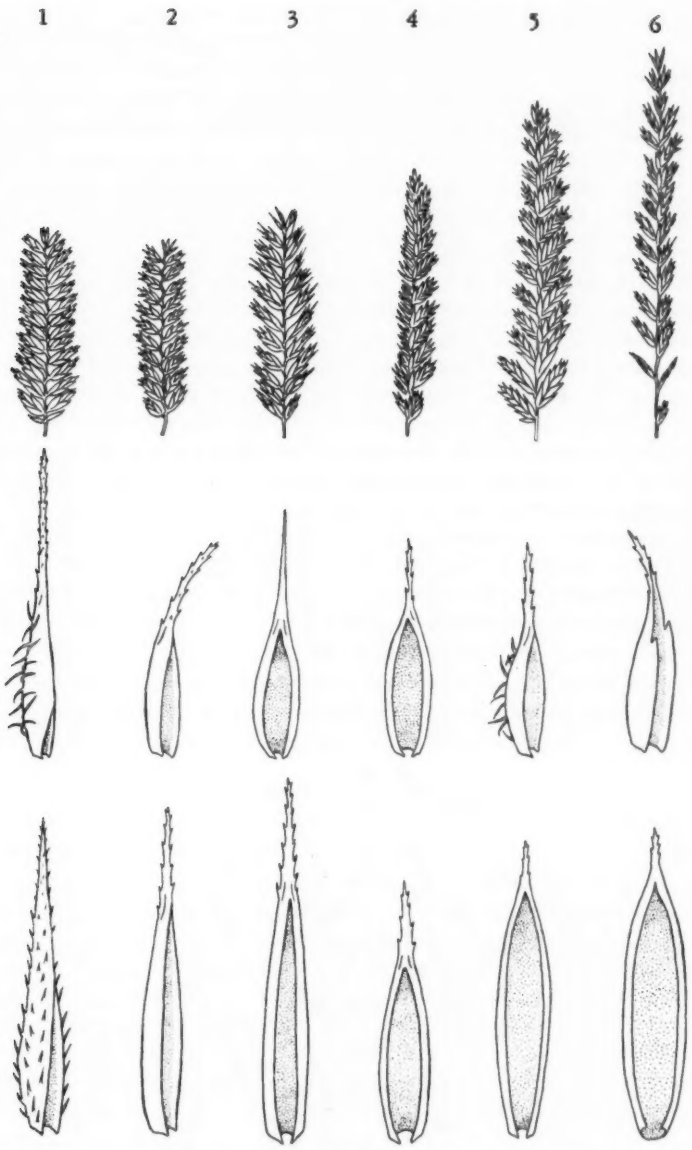
The drawings of both somatic and meiotic chromosomes were made at oil immersion magnification with a camera lucida.

Pollen fertility was determined by staining fresh pollen in a solution of acetocarmine and glycerin (1 : 1).

Cytotaxonomic Considerations

The cultivation of crested wheatgrasses in North America was started in 1906 with seeds introduced from Russia (3, 17). Though the first material came under the names of *Agropyron cristatum* and *A. desertorum*, subsequently these two species together with other allied introductions were lumped together under the name of *A. cristatum*, or crested wheatgrass in the commercial sense. However, at present in the complex of introduced species commonly called crested wheatgrass, at least five taxa can be distinguished according to descriptions given by Nevski in the Flora of the U.S.S.R. (17). These are: *A. cristatum*, *A. pectiniforme*, *A. desertorum*, *A. michnoi*, and *A. sibiricum*. There is considerable confusion regarding the identity of the first two taxa. The distinction of *A. michnoi* from *A. desertorum*, though not very pronounced, can be used for their separate recognition (Figs. 4 and 5). *A. sibiricum*, on the other hand, presents no difficulty in its identification (Fig. 6).

Two commercial varieties of *A. cristatum* s. lat. are grown in the United States and Canada under the names of 'Standard' and 'Fairway' with 28 and



FIGS. 1–6. Drawings of spikes, glumes, and lemmas of some species of *Agropyron*. Top row, spikes. × 0.5; middle row, glumes. × 5; bottom row, lemmas. × 5. Fig. 1. *A. cristatum*. FIG. 2. *A. pectiniforme*. FIG. 3. *A. cristatiforme*. FIG. 4. *A. desertorum*. FIG. 5. *A. michnoi*. FIG. 6. *A. sibiricum*.

14 somatic chromsomes respectively. The name "Standard crested wheatgrass" was first used for material introduced as *A. desertorum* (3). However, *A. desertorum* is quite a distinct species from *A. cristatum*, and within the broad morphological characteristics distinguishing the latter, there are found both a diploid and a tetraploid form which are easily recognizable from each other. To which one of these forms the name *A. cristatum* is correctly applied depends on the identity with Linnaeus's type specimen. Nevski's (17) description of this species refers to the 28 chromosome strain and is being followed in the present paper.

Nevski (17) seems to be inclined to include the 'Fairway' form under *A. pectiniforme*. He also makes *A. dagnae* Grossh. synonymous with it. However, the original description of *A. dagnae* does not fit with that of *A. pectiniforme*. The difference between *A. cristatum* s. str. and *A. pectiniforme* is mainly that the former is hairy in the blade and the spike, while the latter is glabrous (Figs. 1 and 2). Now, there is found a glabrous form which otherwise looks very much like *A. cristatum* and has the same somatic chromosome number of 28. This form is here regarded as *A. pectiniforme*. The validity of this use of the name is open to question and unfortunately the type specimen is of little use in this regard. The chromosome number of *A. pectiniforme* has not been previously reported, owing perhaps to its inclusion under the name *A. cristatum*. In the present study, plants recognized to be morphologically distinct from *A. cristatum* and identified with *A. pectiniforme* were found to have 28 somatic chromosomes (Fig. 7).

It would be valid to attribute specific ranks to the above mentioned taxa only when their genetic interrelationship and mode of evolution were clarified.

FIG. 7. Somatic metaphase of *Agropyron pectiniforme*. × 1675.

Except the 'Fairway' form, all are tetraploids with 28 somatic chromosomes as has been reported by various investigators (1, 2, 6, 15, 20, 21) and verified by the present writer. The chromosomes of all these tetraploids have median or submedian centromeres and lack any secondary constrictions or satellites. As a result, attempts to distinguish them karyomorphologically have not been successful.

The taxon usually named as *A. cristatum* var. 'Fairway' is both morphologically and cytologically different from *A. cristatum* and other related taxa. It is a diploid with 14 somatic chromosomes (6, 21, 23). Plants of 'Fairway' obtained from different sources in North America and Europe were found to be identical in their morphological character (Fig. 3) and chromosome number. That it seems to be the most uniform type in the complex was recognized by all previous workers. Swallen and Rogler (26) reported unsuccessful attempts to hybridize the 28 chromosome 'Standard' type with the 14 chromosome 'Fairway'. They also stated that "variation in growth type and wide taxonomic differences between Fairway and Standard, indicates the need for a separate classification of species." In spite of this, the authors preferred to identify all 'Fairway' plants with *A. cristatum* pending further investigations.

A critical morphological comparison and cytological study performed in the present investigation leaves no doubt regarding the distinctness of the 'Fairway' type from all the other taxa in this group. The only other validly described diploid species of *Agropyron* s. str. is *A. dagnae* Grossh. as reported by Avdulov (2) and Peto (21). Since the 'Fairway' type is morphologically distinct from *A. dagnae*, as well as from all the other species previously described under the genus *Agropyron*, it is necessary to describe it as a separate species. For this taxon the present writer proposes the name *Agropyron cristatiforme*. The description of this species is given below.

Agropyron cristatiforme Sarkar n. sp.

(Synonomy: *Agropyron cristatum* (L.) Gaertn. var. *pectinatum* M.B. (Boiss.); *A. cristatum* commercial variety 'Fairway'.)

Perennis, caespitosa. Culmus 30–40 cm. altus, sub spica villosus. Vagina levis et glabra. Folia angusto-linearis, parum involuta, sparsim hirsuta in utraque facie, cum capillis longioribus in facie superiore, 0.2–0.4 cm. lata, 10–18 cm. longa. Spica densa, sine spatio notabili inter spicula, oblongo-ovata, 4–7 cm. longa, 1.5–2.5 cm. lata, pectinata, rachis villosa. Spicula prope compressa, 3–10 florifera, glabra, 0.7–1.5 cm. longa. Gluma ovato-lanceolata, 0.3–0.4 cm. longa (sine arista; arista fere tam longa quam gluma), naviculara, carinata, glabra, margine albo-membraneo. Lemma lanceolata, subulata, glabra, 0.5–0.7 cm. longa cum arista brevi circa 0.2 cm. longa. Palea lanceolata, 0.4–0.5 cm. longa, bidentata in apice, carina scabra. Anthera longiora dimidio paleae.

Type specimen preserved in National Herbarium of Canada, Ottawa, Canada. (P. Sarkar, No. 47, 1954.)

In order to have a better understanding of this polyploid complex, recognition of morphologically constant forms should be of practical value, whatever the final taxonomic picture. A workable key to the taxa discussed here, based partly on the Flora of the U.S.S.R. (17), is given below.

Spikelets at the nodes of the rachis solitary, sessile, 2–12 flowered; glumes lanceolate or navicular with white, membraneous margins; spikes with a strong, flexible rachis; plants perennial ..... *Agropyron*

(*a*) Spikes short linear or oblong linear, 5–7 cm. long

(*b*) Spikes short linear, indistinctly pectinate, dense without noticeable space between spikelets

Glumes glabrous; culm villous below the spike; blades glaucous, on the upper surface scabrous ..... *A. desertorum*

Glumes ciliate on the keel; culm glabrous; blades on the upper sufrace puberulent ..... *A. michnoi*

(*bb*) Spikes oblong–oval, distinctly pectinate, with squarrose parallel spikelets

(*c*) Spikelets entirely glabrous

Spike dense, but with noticeable space between spikelets; rachis and culm glabrous; blade smooth or scabrous ..... *A. pectiniforme*

Spike dense, with relatively less space between spikelets; rachis villous; culm villous below the spike; blade sparsely hairy ..... *A. cristatiforme*

(*cc*) Spikelets densely hirsute

Spikes very dense, without noticeable space between spikelets ..... *A. cristatum*

(*aa*) Spikes linear, 6–15 cm. long

Sheaths and blades glabrous; lemma acuminate with a very short awn; spike pectinate ..... *A. sibiricum*

Karyotype of *Agropyron cristatiforme*

Unlike that of the tetraploid species, the chromosome morphology of *A. cristatiforme* has been critically studied, since it is the only diploid species of the true crested wheatgrasses available for the present investigation and may possess one of the basic units involved in the evolution of the tetraploid species of this group.

Materials of *A. cristatiforme* from different sources show similar karyotypes. Absolute chromosome lengths could not be used in making the idiogram owing to their variability in different metaphase plates even from the same root tip. However, it was found that the arms of each chromosome maintain a constant ratio in plates of different contraction stages. Also constant is the ratio between the total length of all the chromosomes and that of any

FIGS. 8–9. Karyotype of *Agropyron cristatiforme*. FIG. 8. Somatic metaphase. ×1675. FIG. 9. Idiogram.

one chromosome in a plate. Therefore, in the description of the karyotype, the length of chromosome is expressed as a percentage of the total sum of lengths of all the chromosomes in a plate (relative length), and the ratio of short arm to long arm is used as the index (cf. 27).

Pair I. Long chromosome. Relative length 16.6. Index 0.75. Centromere submedian.

Pair II. Long chromosome. Relative length 16.6. Index 0.85. Centromere submedian.

Pair III. Medium chromosome. Relative length 14.6. Index 0.83. Centromere submedian.

Pair IV. Medium chromosome. Relative length 14.0. Index 0.59. Centromere submedian–subterminal.

Pair V. Medium chromosome. Relative length 14.0. Index 0.95. Centromere submedian.

Pair VI. Medium chromosome. Relative length 13.0. Index 0.46. Centromere submedian–subterminal.

Pair VII. Shortest chromosome. Relative length 11.5. Index 0.68. Centromere submedian–subterminal.

A representative somatic metaphase plate and the idiogram are presented in Figs. 8 and 9. Table I shows the length of each chromosome pair and constituent arms of the somatic metaphase plate drawn.

TABLE I

CHROMOSOME LENGTH IN *Agropyron cristatiforme* (IN MICRONS)

Chromosome pair	Long arm	Short arm	Total length
I	6.5	4.5	11
II	6.2	4.8	11
III	5.0	4.7	9.7
IV	6.5	3.2	9.7
V	4.8	4.6	9.4
VI	6.2	3.2	9.4
VII	4.8	3.5	8.3

Meiosis

Meiotic studies were made on the tetraploid species of *Agropyron* mainly to determine the type of polyploidy. The diploid species, *A. cristatiforme*, was also studied in this connection for a comparison with the tetraploids. Since, in the early period of investigation, the writer was unaware of the distinction between *A. cristatum* and *A. pectiniforme*, the material for meiotic studies on these was mixed. Therefore, this report will be confined to the other three tetraploids, namely, *A. sibiricum*, *A. michnoi*, and *A. desertorum*, together with the diploid, *A. cristatiforme*.

TABLE II

METAPHASE I CONFIGURATIONS IN *Agropyron cristatiforme*

Configuration	Material		
	P-3	P-43	P-55
	No. cells		
7_{II}	520	520	370
$6_{II} + 2_I$	20	14	27
$5_{II} + 4_I$	—	—	2
$1_{III} + 5_{II} + 1_I$	9	—	—
	549	534	399

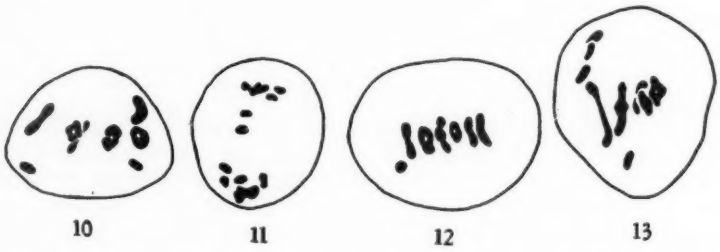
Agropyron cristatiforme

Meiosis in this species was studied in material obtained from three different sources. Table II summarizes the results.

It is evident from the above table that meiosis in all the material is essentially regular. However, an appreciable number of cells showed $6_{II} + 2_I$ at metaphase I (Fig. 10). The univalents are believed to go almost regularly to opposite poles since anaphase I is generally found to be normal. Occasional cells with two lagging univalents (Fig. 11) have been observed in P-3 and P-43. In addition, metaphase I configurations showing $1_{III} + 5_{II} + 1_I$ (Fig. 12) and $5_{II} + 4_I$ (Fig. 13) have been noted in P-3 and P-55 respectively. These configurations indicate probable heterozygosity for segmental interchange.

In spite of irregularities, P-3 and P-43 showed very little pollen sterility, while 55% pollen sterility occurred in P-55. Whether or not this is due to the high frequency of univalents could not be determined.

The chiasma frequency in the material of this species from different sources is shown in Table III.



FIGS. 10–13. Meiosis in *Agropyron cristatiforme*. × 600. FIG. 10. Metaphase I with $6_{II} + 2_I$. FIG. 11. Anaphase I with two lagging univalents. FIG. 12. Metaphase I with $1_{III} + 5_{II} + 1_I$. FIG. 13. Metaphase I with $5_{II} + 4_I$.

TABLE III

CHIASMA FREQUENCY IN *Agropyron cristatiforme*

Material	Total no. chiasmata per cell							Total no. cells	Chiasmata per bivalent
	7	9	10	11	12	13	14		
P-3	1	—	1	13	35	24	—	74	1.72
P-43	—	2	14	40	67	33	2	158	1.68
P-55	—	1	1	7	37	49	20	115	1.80

TABLE IV

METAPHASE I CONFIGURATIONS IN *Agropyron sibiricum* AND *Agropyron michnoi*

A. sibiricum		*A. michnoi*	
Configurations	No. cells	Configurations	No. cells
14_{II}	34	14_{II}	29
$13_{II} + 2_{I}$	11	$1_{IV} + 12_{II}$	34
$1_{III} + 12_{II} + 1_{I}$	17	$2_{IV} + 10_{II}$	43
$3_{III} + 9_{II} + 1_{I}$	1	$3_{IV} + 8_{II}$	4
$1_{IV} + 12_{II}$	11	$4_{IV} + 6_{II}$	19
$1_{IV} + 1_{III} + 10_{II} + 1_{I}$	1	$4_{IV} + 5_{II} + 2_{I}$	1
$3_{IV} + 1_{III} + 6_{II} + 1_{I}$	1	$5_{IV} + 4_{II}$	2
$4_{IV} + 6_{II}$	1	$6_{IV} + 2_{II}$	1
	77		133

Agropyron sibiricum

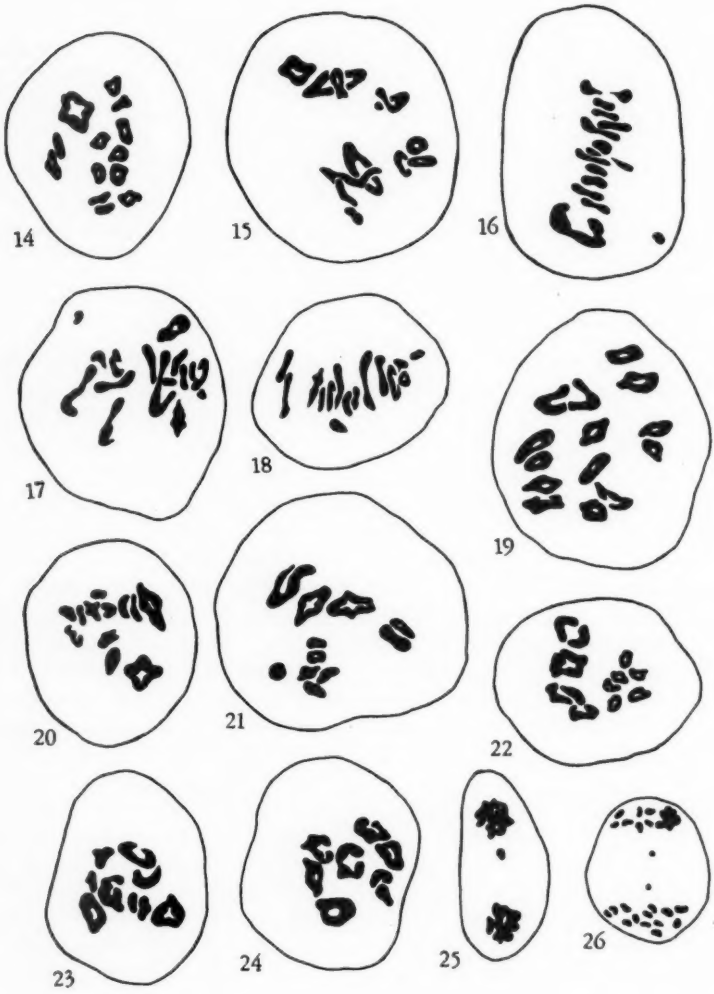
The metaphase I configurations in this species are summarized in Table IV.

The table shows that about 41% of the cells have some multivalents. About 18% of the cells studied showed one or more quadrivalents (Figs. 14–15); 25%, the presence of trivalents (Figs. 15–17); while about 40% had one or two univalents (Figs. 15–18). The maximum number of quadrivalents observed in one cell was four, and that of trivalents, five. Twenty-four per cent of the cells studied showed one trivalent and one univalent in combination with other configurations (Figs. 15–17). This may indicate an autoploid origin followed by some chromosomal rearrangements. In spite of the high frequency of trivalents, the pollen sterility was found to be only 8.5%. The fate of the univalents at anaphase could not be determined with certainty. However of the 93 cells studied at anaphase, five showed a lagging univalent at the middle of the spindle which presumably divides, with the daughter chromatids going to opposite poles, since no micronucleus was observed.

Agropyron michnoi

Meiotic studies in this species have been confined to the analysis of metaphase I configurations. The results are summarized in Table IV.

It can be seen from the above table that quadrivalents are found in about 77% of the cells studied. Of these, cells with two quadrivalents and 10



FIGS. 14–26. Meiosis in some tetraploid taxa of *Agropyron*. × 600. FIGS. 14–18. *A. sibiricum*. Metaphase I cells with: FIG. 14. $1_{IV} + 12_{II}$; FIG. 15. $3_{IV} + 1_{III} + 6_{II} + 1_{I}$; FIG. 16. $1_{III} + 12_{II} + 1_{I}$; FIG. 17. $3_{III} + 9_{II} + 1_{I}$; FIG. 18. $13_{II} + 2_{I}$. FIGS. 19–24. *A. michnoi*. Metaphase I cells with: FIG. 19. $1_{IV} + 12_{II}$; FIG. 20. $2_{IV} + 10_{II}$; FIG. 21. $3_{IV} + 8_{II}$; FIG. 22. $4_{IV} + 6_{II}$; FIG. 23. $5_{IV} + 4_{II}$; FIG. 24. $6_{IV} + 2_{II}$. FIGS. 25–26. *A. desertorum*. Anaphase I cells with: FIG. 25. A lagging univalent. FIG. 26. A dividing univalent.

bivalents (approximately 32%) are most frequent. In addition, approximately 14% of the cells showed four quadrivalents and six bivalents. Metaphase I cells containing different numbers of quadrivalents are shown in Figs. 19–24. Meiosis in this species, therefore, presents a clear indication of autoploidy. If this is autotetraploidy, the maximum number of quadrivalents expected per cell would be seven. This is approximated in one cell observed with six quadrivalents and two bivalents (Fig. 24).

In spite of the irregular pairing of chromosomes in metaphase I, the pollen sterility in this species was found to be only 10%. This indicates that perhaps in most cases the quadrivalents disjoin alternately at anaphase with equal distribution of the component chromosomes to the opposite poles.

Agropyron desertorum

The association of all the chromosomes at metaphase I could not be definitely discerned in this species. However, one (very rarely two) distinct univalent could be clearly distinguished in as many as 80% of the metaphase I cells studied. Anaphase I was better suited for observation and about 40% of the cells studied at this stage showed lagging univalents near the equatorial plate (Fig. 25). It seems that this univalent either divides, its daughter chromatids going to opposite poles (Fig. 26), or forms a micronucleus. In spite of the high frequency of univalents, the pollen sterility in this species was found to be only about 12%.

A high frequency of univalents has been observed in crested wheatgrasses by Myers and Hill (15). They reported also an average number of 3.7 quadrivalents per cell. However, the taxonomical identity of their material could not be ascertained. Thus, a comparison of the present data with those of Myers and Hill is not possible.

Discussion

Morphological and cytological data so far available seem to show that the placing of the crested species of wheatgrasses in a group separate from the other species provides a reasonable basis for the understanding of their systematic status and mode of evolution. It must be mentioned in this connection, however, that comprehensive cytogenetical data to support the recent classifications of the wheatgrasses are not yet available. Although little more than 10% of the species have been studied cytologically, it is known that members of this group of grasses are characterized by having large chromosomes with seven as the basic number. It is also a well-known fact that absolute barriers of incompatibility are not typical for the tribe as a whole, and that the evolution of many of the taxa is based on autoploidy or alloploidy involving one or more diploid species. Stebbins and Pun (25), from their study of a group of diploid species, observed that a particular genome consisting of chromosomes essentially homologous to those of diploid species is widely distributed both at the diploid and the polyploid level in the wheatgrasses. This indicates the importance of a thorough understanding of all the diploids in this group.

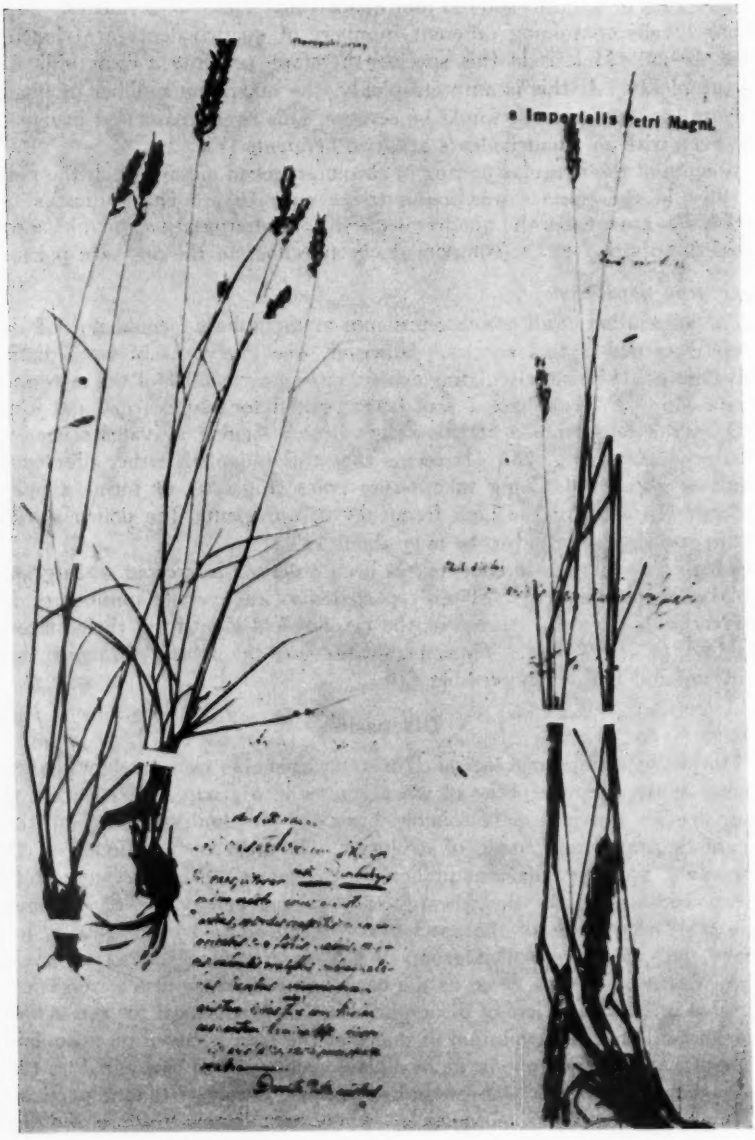


Fig. 27. Type specimen of *A. desertorum*.

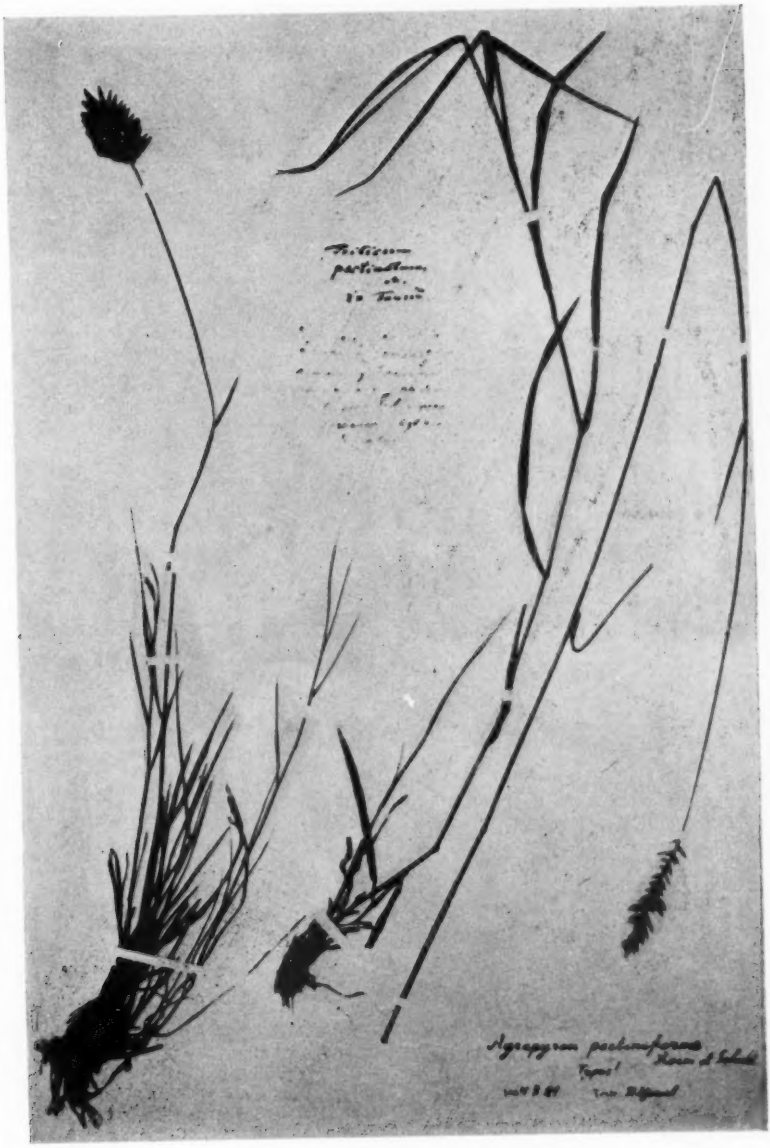


FIG. 28. Type specimen of *A. pectiniforme*.

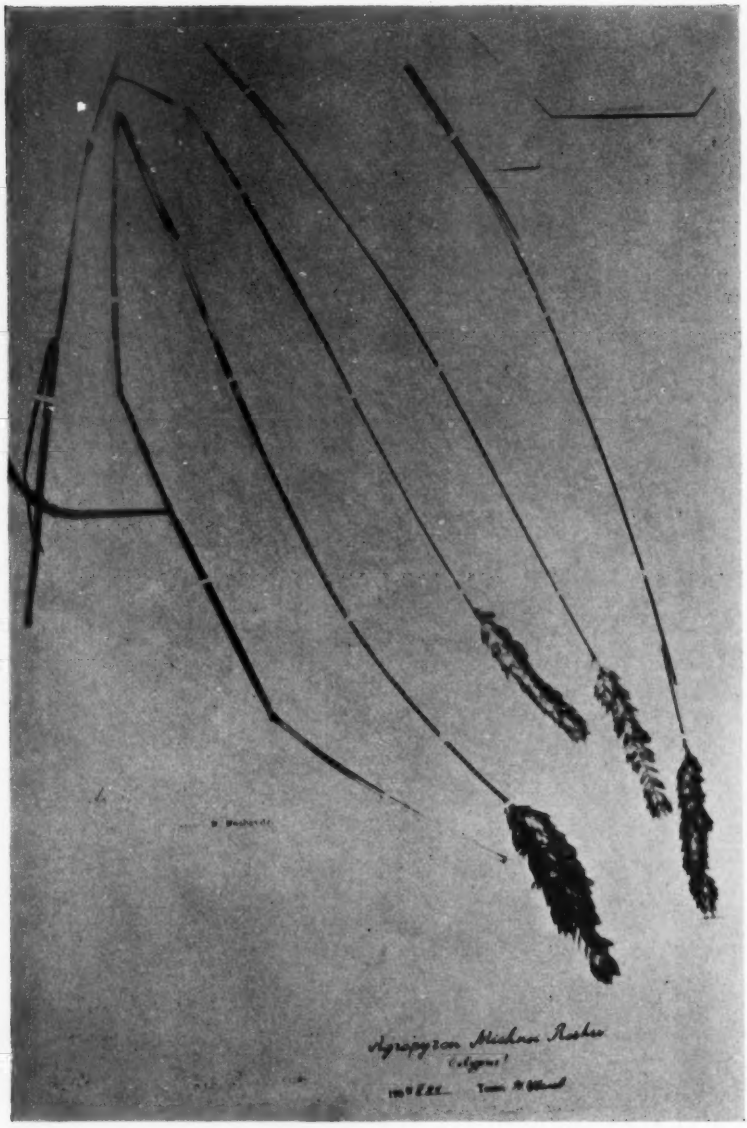


FIG. 29. Cotype specimen of *A. michnoi*.

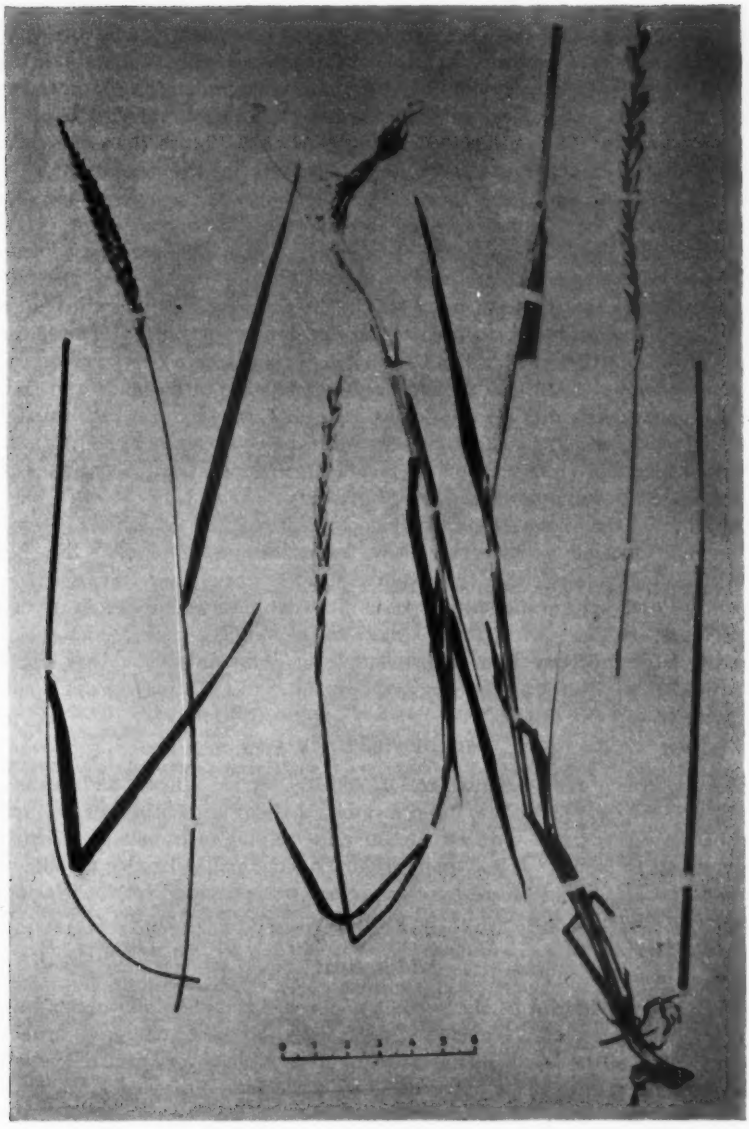


FIG. 30. Specimen of *A. sibiricum* (from former herbarium of Willdenow).

Two of the tetraploid taxa in which studies of meiosis are reported here—*Agropyron michnoi* and *A. sibiricum*—show indications of autoploid derivation. Autoploidy has also been reported in *A. cristatum* by Favorsky (4). It thus appears that evolution in this group of grasses has been mainly through autoploidy followed by structural and genic changes in the chromosomes. The range of morphological variation, though not very great, can hardly be accounted for by differentiation of a single genome. More than one closely related diploid taxon must have been involved in the evolution of the tetraploids. Therefore, the type of polyploidy that has taken place here can be termed hemi-autoploidy in the sense of Löve and Löve (12) or segmental alloploidy in the sense of Stebbins (24). The similarity in morphology and the constant occurrence of multivalents in meiosis of the tetraploids indicates that these taxa are still in the process of active evolution and have not differentiated enough to attain cytogenetic stability. The natural areas of distribution of almost all the taxa discussed here overlap with each other in the Soviet Union. Thus, for the emergence of constant and stable types, long periods of time would be necessary except when facilitated by artificial isolation and selection for constant types. Evidently the taxonomic status of such morphological entities is difficult to decide until all the ancestral diploids are known and the process of evolution clarified.

Apart from the above evolutionary considerations, the necessity of a closer taxonomic revision of the so-called "intraspecific polyploids" has been emphasized in recent times by Knaben (8), Valentine (29), Löve (11), and others. Löve (11) questioned the possibility that taxa with different chromosome numbers exist without any morphological distinction. The case of *Agropyron cristatiforme* is a good example of a diploid taxon that can be separated from its tetraploid relatives by small but distinct morphological characters.

Acknowledgments

The writer is greatly indebted to Dr. A. Löve for the interest he has shown in the present work, his advice, and inestimable help in securing seed samples and herbarium specimens; to Dr. D. Löve for helping in translating scientific papers; and to his wife Mrs. Nina Sarkar for technical assistance throughout the work. The financial aid for the project was provided by the National Research Council of Canada in the form of a grant (PR 78).

Addendum

When the manuscript of the present paper was complete and about to be sent for publication, the November, 1955, issue of the Canadian Journal of Botany came to the hands of the writer. It contained a paper* relevant to the present account. This paper deals with the same group of taxa as that in the present paper. However, identification of plants recognized as *A. michnoi* by Knowles seems to be doubtful (cf. Fig. 29). From the published

*Knowles, R.P. *A study of variability in crested wheatgrass.* *Can. J. Botany, 33: 534-546. 1955.*

photographs and morphological characteristics, it appears to the present writer that the plants concerned are 28 chromosome forms of *A. cristatum*. This would also explain the reported ease of crossing between this form and the 14 chromosome 'Fairway' form.

In view of the difficulty in identifying different taxa of crested wheatgrass, some available photographs of type and cotype specimens are given here (Figs. 27–30).

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CONTRIBUTION TOWARDS A MONOGRAPH OF THE GENUS STELIS SW.[1]

By Leslie A. Garay[2]

Abstract

The various categories within the genus Stelis are discussed. In Part I the history of the genus is given as well as an analysis of the Lindleyan classification which is shown to be artificial. A system of classification is proposed using what appears to be more constant and therefore more significant characteristics. In Part II new species are described and arranged according to the proposed system. These species are as follows: *Stelis convallarioides* sp. nov.; *Stelis Jamesoni* Lindl. var. *parviflora*, var. nov.; *Stelis papillosa* sp. nov.; *Stelis cochlearis* sp. nov.; *Stelis navicularis* sp. nov.; *Stelis rhomboidea* sp. nov.; *Stelis sanguinea* sp. nov.; *Stelis genychila* sp. nov.; *Stelis tenuipetala* sp. nov.; *Stelis cryptochila* sp. nov.; *Stelis violascens* sp. nov.; *Stelis ecallosa* sp. nov.

Part I

Introduction

Perhaps no other genus in the family Orchidaceae presents as many difficulties in species identification, as does the genus *Stelis*. Primarily this is due to the extreme minuteness of the flowers which are of pinhead size in most of the species. This genus is in urgent need of critical field observation, since herbarium material presents an exasperating number of perplexities. Grave problems are met when the material at hand has to be determined solely with the aid of the original descriptions written with telegraphic brevity by the earlier authors, such as the following for *Stelis triplicata* Lindley: "*f. oblongo, sess. caule longiore, spica duplo longiore late disticha, ss. triangulis confluentibus ad sinus plicatis, pp. trunc. dorso gibbosis, lab. minore truncato*". When this description was prepared, the genus consisted of 133 species. Now around 500 different taxa are known, and I can safely say that the cited description characterizes at least 20 to 30 of them.

Since such difficulties are recognized generally, I decided to study the whole genus monographically in the hope of eliminating the problems now involved in delimiting almost every species. For a long while it appeared that I might have to give up this aim, since the majority of the earlier taxa are known only from type collections to which I had no access. I refer here to Lindley's invaluable collection kept at Kew Herbarium. No material of the Lindleyan types could be obtained on loan nor was it possible to secure photographs of these from Kew. A real understanding of the problem was shown by Dr. K. Rechinger, Director of the State Museum of Natural History, Vienna, Austria, where the Reichenbach orchid herbarium is preserved. All *Stelis* material

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(over 1000 sheets) was transported across the sea and with it came the solution to the telegraphic descriptions since, besides the nomenclatorial types of species described by Reichenbach, all Lindley's species were included, represented either by duplicates of his specimens or by a life-sized tracing of them supplemented with flowers taken from the original material.

Many herbaria have been consulted during the course of this study and these will be enumerated later. However, special attention must be paid here to the Orchid Herbarium of Oakes Ames at Harvard University which is the richest in types and the most complete on our continent. It supplied invaluable information about species described after Lindley and Reichenbach ceased to be active.

A general picture of the genus, the result of several years' study, is presented here. A detailed monograph furnished with keys and full descriptions of all known species will follow within a reasonable time. This paper is limited to the discussion and description of the new categories proposed within the genus itself as well as to descriptions of the newly recognized taxa.

History of the Genus

The genus *Stelis* was established by the Swedish botanist Olof Swartz (10) with two species, viz. *S. ophioglossoides* and *S. micrantha*. Swartz based his *S. ophioglossoides* on the taxon described and illustrated originally as *Epidendrum ophioglossoides* by Jacquin (5). The genus immediately was taken up by such noted authors of that time as Humboldt, Hooker, Poeppig, and Lindley, all of whom made additions to it and by 1840 the number of species had increased to 25. As a result of a large number of expeditions to the American tropics, which sought orchids primarily for commercial purposes, a large number of botanical species, including numerous *Stelis*, have reached the European taxonomists. The increase was on such a large scale that within 20 years it made necessary the publication of a monograph-like treatment of the genus (7). Lindley in his *Folia Orchidacea Stelis* enumerated 133 different species, all of them characterized with the brevity previously referred to and from that time on, Reichenbach, Rodrigues, Kraenzlin, Schlechter, Ames, and Schweinfurth were the important contributors. With the exception of Professor Ames, who undertook a critical study of certain groups of related species of Central American origin (1), these authors merely described new species. The South American species, especially those of the Andean region, have never been the subject of special study by anyone, in spite of the fact that the Andean region must be considered the center of distribution for the genus. The number of known distinct species may be estimated at around 500 and many more may be expected if the botanists of that region will give more attention to these highly interesting members of our tropical vegetation.

The Earlier Classifications

The first and only attempt to divide the genus into sections was made by Lindley (7) as follows:

"Calyx subaequaliter tripartitus ..... Sect. I. EUSTELIS.
Bractae magnae distiche imbricatae ..... A. *Distichae.*
——— parvae vix imbricatae
Racemi plures ..... B. *Polystachyae.*
——— solitarii ..... C. *Monostachyae.*
Calyx calvus, caulis vix ullus ..... 1. apodae
Calyx calvus, caulis maturo folio vix longior ..... 2. brachypodae
* Labellum truncatum
** ——— planum aut cavum indivisum
*** ——— tridentatum vel rhombeum
Calyx calvus, caulis maturus folio multo longior ..... 3. macropodae
Calyx intus pilosus ..... 4. barbatae
Calyx manifeste irregularis,
bilobus (sep. lat. maxima pro parte connatis) ..... Sect. II. DIALISSA.
bilabiatus (sep. lat. subliberis, supremo crassiore multo minoribus) ..... Sect. III. LABIATAE"

This arrangement shows a system which may not easily be attacked. As far as the main sections are concerned, the basic principles must continue to be maintained, although with some modification. Certainly the system appeared to be practicable at the time it was proposed, but it proved to be far from natural, since its chosen characteristics may place very closely related species in different subsections.

The section EUSTELIS, where the sepals are equal in size and similar in shape, includes 80% of the described species. The way Lindley tried to break up this section into subsections and further categories is very unfortunate since the characters employed have proved to be highly inconstant.

The subsection *Distichae*, in which Lindley included nine species, when taken in its strict sense can incorporate only two species, viz. *S. disticha* Poepp. & Endl. and *S. triplicata* Lindl. Only these two species have large, distichously imbricate bracts. The bracts in *S. spathulata* Poepp. & Endl. can not be called "magnae" because they are only 2–3 mm. long. Likewise, on the type specimen as well as on several additional collections, I have observed that the bracts tend to be imbricate while the inflorescence is still developing and that the lowest bracts may enclose fully matured flowers while the upper ones are just beginning to form. This observation is applicable to *S. foveata* Lindl., *S. muscifera* Lindl., *S. ochreata* Lindl., and *S. glumacea* Lindl. Though some of these species have essentially large bracts, they are not imbricate on the mature inflorescence. *Stelis attenuata* Lindl. which is also referred to this subsection has a spirally arranged inflorescence, far from distichous.

The next two subsections, *Polystachyae* and *Monostachyae*, together with four subcategories of the latter, are based on characters which are exceedingly variable, not only in this genus, but also in the whole subtribe *Pleurothallideae* of which *Stelis* is an outstanding member. First, whether a plant produces one or more inflorescences is often governed by physiological or ecological factors, and it may have inflorescences produced in succession (succedaneus).

Further, the comparative length of the secondary stem and the leaf as well as the length of the inflorescence and the subtending leaf are characters of individual plants rather than of species, since they are very responsive to different environmental influences. In these two sections then the only usable differentiating characters are those of the lip, about which more will be said shortly.

The section DIALISSA, as proposed by Lindley, represents the most unnatural category of the three. Lindley failed to include in his key the subsections within Dialissa, but he divided it, as he did Eustelis, into *Distichae*, *Polystachyae* and *Monostachae*. The value of these categories has already been discussed.

The type of the section DIALISSA is *Dialissa pulchella* Lindl., (6) now called *Stelis Dialissa* Rchb.f. (3). This is the only species in the genus where the three sepals are adnate to each other more than half of their entire length, giving the calyx an urceolate appearance. The other species referred here do not exhibit this character. Such characteristics as the almost complete adnation of lateral sepals or the almost complete lack of such fusion (section LABIATAE) are constant features which should be employed in differentiation of groups of species.

On the other hand, Reichenbach recognizes but two sections, DISEPALAE and TRIVALVES (9), the former encompassing Dialissa and Labiatae and the latter synonymous with Eustelis. Disepalae and Trivalves are nomina nuda since they are merely headings without descriptions, though they were obviously intended to replace Lindley's names. Reichenbach's sectional names never were taken up by later authors. Cogniaux uses Lindley's original treatment (4). Bentham and Hooker (2) as well as Pfitzer (8) recognize but two sections, viz. Eustelis and Dialissa citing Trivalves and Disepalae in synonymy. To unite Dialissa and Labiatae under one section is hardly permissible and creates only additional confusion.

Reclassification

It is obvious that vegetative characters can not be employed in differentiation of various groups because they are highly variable in any given species. The only characteristics which can be used to delimit species so that the relationships among them are clear are those of floral structure. Lindley pointed out (7) that among the 133 species known to him, he could find only 6 which showed a certain deviation from the common type in the form of the column. This number has not increased in proportion with the numbers of new taxa. Therefore, the shape of the column is at best of only very restricted use in speciation.

In general three distinct types of floral structure can be segregated on the basis of the floral calyx: (1) The flowers are rotate. All sepals are similar in shape, equal in size, and free from each other almost to the base. (2) The flowers are ringent. The dorsal sepal differs from the lateral ones in shape

and often in size. The lateral sepals may be shortly connate above the base or are united to their tips. (3) The flowers are urceolate. The three sepals through lateral adnation form a distinct sepaline tube.

The first main group with rotate flowers includes a vast number of species which offer additional characters constant in, and common to, a large number of taxa, that is, the characteristics of the lip. In type I, the lip is entire, and broader than long. Its callosity is always transverse and biparted, observable at any point between the base and the tip. In type II, the lip is longer than broad, its apex truncate with minute apiculum; or, if the apiculum is not present it is replaced by a shallow notch, i.e. the apex is retuse. The callosity is either undivided at the base of the lip or distinctly bilamellate, in which case the individual lamellae are inserted obliquely above the base. In type III, the lip is distinctly three-lobed or has a denticulate apex.

It should be mentioned that Reichenbach (9) had a somewhat similar approach in his section Trivalves. He grouped 18 species in eight sections on the different shapes of the lip. Undoubtedly, these characters are specific rather than sectional and, furthermore, the categories do not correspond to the species he selected for them. The first section in the Trivalves, characterized by a "*Labellum integrum transversum retusum*", contains *S. major* Rchb.f. and *S. grandis* Rchb.f. His own description of *S. grandis* (3) shows it to be of an entirely different nature: "*Labello flabellato antice bisinuato hinc trilobulo*", which eliminates the possibility of a retuse lip. Since similar mistakes are to be found throughout the rest of his interpretation, I am forced to ignore his groupings entirely.

The second main group is composed of species whose flowers are ringent. The basic principles here are those originally suggested by Lindley. The dorsal sepal and the lateral sepals differ from each other in shape and generally in size. The character of basic importance in this group is the adnation of the lateral sepals and, using this criterion for segregation, two categories can be recognized: the first, in which the lateral sepals are more or less free from each other, and the second in which they are united into one body.

The third main group is characterized by urceolate flowers. The highly diverse *S. Dialissa* is the only species which belongs here. Apparently, this taxon is very different from the rest of the Stelides but the structure of its column maintains it in the genus. *Stelis Dialissa* is a borderline species through which the relationship of the genus *Stelis* to other genera in the Pleurothallideae may be traced.

The following descriptions are given in accordance with the International Rules of Botanical Nomenclature to characterize the selected categories as indicated in the above discussion.

Subgenus STELIS

Flores rotatae. Sepala similia inter se conformia. Labellum sive omnino integrum sive trilobatum seu denticulatum. Callus labelli maxime variabilis.

Typus: *Epidendrum ophioglossoides* Jacq.

Subgenus INAEQUALES

Flores ringentes. Sepalum posticum a sepalis lateralibus valde diversum. Sepala lateralia inter se aut maxima pro parte libera aut valde connata.

Typus: *Stelis purpurea* Willd.

Subgenus DIALISSA

Flores urceoliformes. Sepalum dorsale cum sepalis lateralibus altius connatum, superne semiapertum, basin versus ventricoso-inflatum.

Typus: *Dialissa pulchella* Lindl.

Conclusion

As it has been shown, the only characteristics which may be selected to segregate various groups of species and, at the same time, reflect the relationships among them are those of the flowers. These characters are: the comparative length and shape of the sepals and the form and callosity of the lip. In translating these characters into a systematic order the following key is presented, which also provides a summary for this paper.

A. Sepala similia, inter se conformia ..... Subgen. STELIS
  a. Labellum omnino integrum
    b. Labellum papulosum, transversum, i.e. latius quam longius; calli disci transversi, bipartiti, in vario modo dispositi ..... Sect. Papulosae
    bb. Labellum concavum, plusminusve ovatum, i.e. longius quam latius; calli disci integri sic basali vel lamelliformes sic lateraliter oblique inserti ..... Sect. Concavae
  aa. Labellum trilobatum vel antice denticulatum ..... Sect. Distinctae

AA. Sepala dissimilia, i.e. sepalum dorsale a sepalis lateralibus valde diversum
  a. Sepalum dorsale cum sepalis lateralibus brevius connatum; flores ringentes ..... Subgen. INAEQUALES
    b. Sepala lateralia maxima pro parte libera ..... Sect. Fissae
    bb. Sepala lateralia valde connata ..... Sect. Valvae
  aa. Sepalum dorsale cum sepalis lateralibus altius connatum, urceolum formans ..... Subgen. DIALISSA

Part II

During the course of this study several new species were recognized. The description of these are arranged according to the new system proposed above.

Subgenus STELIS, Section Papulosae

Stelis convallarioides Garay, sp. nov. Fig. 1A

Epiphytica, usque ad 30 cm. alta; radicibus filiformibus, glabris; caulibus caespitosis, vaginis ternis dimidium usque obtectis, 4–5 cm. longis; foliis obovatis, obtusis vel acutis, in petiolum 2 cm. longum angustatis, 8–9 cm. longis, 2.5–3 cm. latis; inflorescentiis singulis, arcuatis, folia multoties longioribus, usque ad 25 cm. longis; bracteis tubulosis, pedicelli multoties brevioribus; floribus satis magnis, leviter pendulis, glabris; sepalis inter se simillimis, late triangulis, acutis, 5-nerviis, 5 mm. longis et latis; petalis

carnosis, reniformibus, 3-nerviis, 1 mm. longis, 1.5 mm. latis; labello carnoso, antice excavato, in medio incrassatione transverse donato, 0.75 mm. longo, 1 mm. lato; columna humili; ovariis longe pedicellatis, cum pedicellis 5 mm. longis.

Colombia: Intendencia del Choco; Tutunendo, 20 km. north of Quibdo. Alt. 80 meters. May 19–20, 1931. Coll. *W. A. Archer No. 2143.* Type in the herbarium of the United States National Museum, Washington, D.C., No. 1519162.

Stelis Jamesoni Lindl. var. ***parviflora*** Garay, var. nov. Fig. 1B

A typo in habitu graciliori et floribus minoribus differt. Epiphytica, usque ad 25 cm. alta; rhizomate breviter ascendenti; caulibus secundariis 3–4 cm. longis; foliis obovatis, acutis vel obtusis, longe petiolatis, 7 cm. longis, 1 cm. latis, petiolo 2 cm. longo; inflorescentiis folia multo superantibus, gracillimis, dense multifloris; floribus pallide roseolis, illis typi simillimis sed duplo minoribus, 4 mm. in diam.

Colombia: Dept. of Cundinamarca; Cordillera Oriental. San Isidro, 7 km. south of Gachala. Alt. 2200 meters. May 27, 1944. Coll. *M. L. Grant* and *F. R. Fosberg No. 9362.* Type in the author's herbarium No. 3104. Isotype in the herbarium of the U.S. National Museum.

Stelis papillosa Garay, sp. nov. Fig. 1C

Epiphytica, ascendenti, usque ad 35 cm. alta; radicibus filiformibus, satis longis, flexuosis, glabris; caulibus ascendentibus, in medio univaginatis, usque ad 15 cm. longis; vagina ad 8.5 cm. longa, ampla, apice obliqua, acuta; foliis carnosis, ovato-oblongis, acutis, basin versus abrupte in petiolum 1.5–2 cm. longum angustatis, cum petiolo usque ad 15 cm. longis, 2.5 cm. latis; inflorescentiis plurimis (1–4), folia dimidium aequantibus seu brevioribus; bracteis obliquis, distichis, tubulosis, acutis, 2–3 mm. longis; floribus rotatis, atropurpureis; sepalis inter se simillimis, semirotundis, acutis, trinerviis, intus extusque papillosis, 2 mm. longis et latis; petalis minutis, reniformibus, concavis, apice incrassatis, papillosis, 0.75 mm. longis, 1.25 mm. latis; labello cordato-reniformi, apice cucullato, externo papilloso, disco cum callo bipartito papulosoque ornato, 1 mm. longo, 1.2 mm. lato; ovariis cylindraceis, pedicellis aequilongis.

Ecuador: Prov. Tungurahua, vicinity of Patate, hacienda Leito. Alt. 2800 meters. Aug. 5, 1939. Coll. *Erik Asplund No. 8090.* Type in the herbarium of the State Museum of Natural History, Stockholm, Sweden.

SECTION Concavae

Stelis cochlearis Garay, sp. nov. Fig. 1D

Epiphytica, 18 cm. alta; radicibus filiformibus, flexuosis, glabris; rhizomate ascendenti, lignoso; caulibus secundariis oblique ascendentibus, cum vaginis ternis, amplis omnino obtectis, ad 7 cm. longis; foliis obovato-ligulatis, valde coriaceis, acutis, basin versus attenuatis, 7–8 cm. longis, 0.8–1 cm. latis;

inflorescentiis folia superantibus, singulis, breviter pedunculatis, dense multifloris; bracteis navicularibus, acuminatis, distichis, flores duplo superantibus, 5 mm. longis; floribus valde carnosis seu coriaceis, ut videtur cleistogamis; sepalis inter se simillimis, in tubum 1 mm. longum connatis, triangulis, margine involutis, 3-nerviis, 2 mm. longis et latis; petalis minimis, transversis, carnosis, 3-nerviis; labello maximo, valde cochleato, suborbiculari, apiculato, basin callo lamelliformi, erecto, antice bifido ornato, petala duplo latiori; columna humili, brachiis magnis, rostello maximo; ovariis brevissime pedicellatis.

Colombia: Dept. Antioquia; en rocas en el Balkeron de Sonson. Alt. 2700 meters. Aug. 20, 1948. Coll. *W. Johnson & F. A. Barkley No. 18C804* (Type); also No. 18C798. Type in the herbarium of the U.S. National Museum No. 2102700.

Stelis navicularis Garay, sp. nov. Fig. 1E

Epiphytica, caespitosa, usque ad 30 cm. alta; radicibus fasciculatis, filiformibus, flexuosis, glabris; caulibus erectis, in medio univaginatis, ad 16 cm. longis; foliis coriaceis, ovato-oblongis, acutis vel obtusis, basin in petiolum 1 cm. longum augustatis, cum petiolo ad 12 cm. longis, 2 cm. latis; inflorescentiis heterochronicis, 1–4-nis, leviter arcuatis, folia sensim superantibus, dense multifloris; bracteis infundibularibus, oblique truncatis, acutis, glabris; sepalis inter se aequalibus, ovatis, acutis, 3-nerviis, intus densissime papillosopuberulis, 3 mm. longis, 2.75 mm. latis; petalis transversis, apice incrassatis, leviter cucullatis, 3-nerviis, 1 mm. longis, 1.5 mm. latis; labello naviculari, valde concavo, subrotundo, antice apiculato, basin toris 2, leviter divergentibus ornato, 1 mm. longo et lato; columna humili, brachiis late ovatis, obtusis; ovariis cum pedicellis bracteas duplo longioribus.

Colombia: Dept. de Caldas; Cerro Churumbelo, second ridge north of Quebrada Penas Blancas, southwest of Santuario, Cerro Tatama. Alt. 2835 meters. Dec. 3, 1944. Coll. *H. St. John No. 20.875.* Type in the author's herbarium No. 3363. Isotype in the herbarium of the U.S. National Museum.

Stelis rhomboidea Garay, sp. nov. Fig. 1F

Epiphytica, usque ad 15 cm. alta; radicibus filiformibus, flexuosis, glabris; caulibus breviter ascendentibus, 5–6 cm. longis; foliis oblongis, obtusis, distincte petiolatis, petiolo incluso 4 cm. longis, 1 cm. latis; inflorescentiis gracillimis ad 12 cm. longis; bracteis cucullato-triangulis, 1–2 mm. longis; floribus remotis, atropurpureis; sepalis inter se aequalibus, lanceolatis, 3-nerviis, glabris, 3 mm. longis, 1.25–1.5 mm. latis; petalis minutis, orbicularibus, apice leviter incrassatis, uninerviis; labello rhombeo, antice leviter excavato, disco mediano cum lamellis binis, parallelis ornato; columna humili, brachiis rotundatis; ovariis cylindraceis, pedicellis aequilongis.

Columbia: Dept. de Cundinamarca; Cordillera Oriental, Paramo de Guasca. Alt. 2840 meters. June 2, 1940. Coll. *J. Cuatrecasas No. 9465.* Type in the herbarium of the U.S. National Museum No. 1850742.

SUBGENUS INAEQUALES, SECTION Fissae

Stelis sanguinea Garay, sp. nov. Fig. 2A

Epiphytica, ascendenti, usque ad 25 cm. alta; radicibus filiformibus, flexuosis, glabris; caulibus primariis ascendentibus, caulibus secundariis in angulum acutum suberectis, simplicis vel proliferis cum vaginis ternis papiraceis, obliquis, acutis obtectis, 7 cm. longis; foliis oblongis, acutis vel obtusis, basin in petiolum 2–2.5 cm. longum angustatis, petiolo incluso 10 cm. longis, 1.5–2.5 cm. latis; inflorescentiis singulis, usque ad basin floriferis, folia valde superantibus, ad 15 cm. longis; bracteis lanceolatis, brevissime tubulosis, acuminatis, atrosanguineis, 8 mm. longis; floribus maximis, atrosanguineis; sepalo postico ovato-lanceolato, acuto, margine ciliolato, intus papilloso, 7-nervio, 10 mm. longo, 5 mm. lato; sepalis lateralibus breviter connatis (in natura usque ad apicem connatis), ovato-lanceolatis, acutis, distincte ciliolatis, intus papillosis, 5-nerviis, 10 mm. longis, 4 mm. latis; petalis transversis, obtusis, margine leviter incrassatis, 3-nerviis, 1.25 mm. longis, 2mm. latis; labello e cuneato basi abrupte dilatato, quasi unguiculato, reinformi, apicem versus excavato, basin cum callo bipartito ornato, 1.5 mm. longo et lato; columna humili, bibrachiato, brachiis obtusis; ovariis cylindraceis cum pedicellis bracteas longioribus.

Colombia: Dept. de Caldas; Cordillera Occidental. Cerro Churumbelo, second ridge north of Quebrada Penas Blancas, southwest of Santuario, Cerro Tatama. Alt. 3135 meters. Dec. 3, 1944. Coll. *H. St. John No. 20878*. Type in the author's herbarium No. 3358. Isotype in the herbarium of the U.S. National Museum.

Stelis genychila Garay, sp. nov. Fig. 2B

Epiphytica vel saxicola, usque ad 25 cm. alta; radicibus fasciculatis, filiformibus, glabris; caulibus erectis satis crassis, in medio univaginatis, ad 10 cm. longis; foliis semicoriaceis, oblongis, obtusis vel subacutis, basin breviter petiolatis, usque ad 12 cm. longis; inflorescentiis folia multo superantibus, usque ad basin densissime floriferis; floribus satis magnis, 9 mm. in diam.; sepalis inter se valde connatis; sepalo postico ovato, acuto, 3-nervio, 5 mm. longo, 3 mm. lato, pars libera 3 mm. longa; sepalis lateralibus a sepalo postico valde diversis, oblique suborbicularibus, acutis vel brevissime acuminatis, 3-nerviis, 4.5 mm. longis latisque; petalis transversis, apice carnosis, 3-nerviis, 1.5 mm. longis, 2.5 mm. latis; labello genyculato, ovato, obtuso, apice leviter cucullato-excavato, ad plicam callo incrassato, bipartito ornato; ovariis cylindraceis, bracteas tubulosas aequilongis.

Colombia: Distr. Cauca, ad pag. El Tambo, in silva ad Munchique. Alt. 2300 meters. Oct. 24, 1936. Coll. *K. v. Sneidern No. 1095*. Type in the herbarium of the State Museum of National History, Stockholm, Sweden.

Stelis tenuipetala Garay, sp. nov. Fig. 2C

Epiphytica, caespitosa, usque ad 20 cm. alta; radicibus filiformibus, satis numerosis, fasciculatis, leviter flexuosis, glabris; caulibus secundariis erectis, cum vaginis ternis, arcte adpressis, omnino obtectis, 5–8 cm. longis; foliis

oblongis, obtusis, basin versus in petiolum canaliculatum attenuatis, satis coriaceis, cum petiolo, 2 cm. longo, ad 9–10 cm. longis, 0.8–1 cm. latis; inflorescentiis, a spatha 8 mm. longa, lateraliter compressa, singulis bis ternis, folia multoties brevioribus; bracteis distichis, tubulosis, oblique-acutis, brevissime apiculatis, pedicellos aequilongis vel sensim brevioribus, 1.25 mm. longis; floribus minutis, diaphanis; sepalo postico oblongo, acuto, cum sepalis lateralibus distincte connato, 3-nervio, 2 mm. longo, 1.5 mm. lato; sepalis lateralibus ovatis acutis, 3-nerviis, dorsali majoribus, 3 mm. longis, 2 mm. latis; petalis valde tenuosis, fere orbicularibus, uninerviis, margine superiori sensim incrassatis, 0.75 mm. longis et latis; labello cochleato-trilobo, lobis lateralibus rotundatis, lobo intermedio angustissime triangulo, acuminato; disco 3-nervio, ceterum nudo, 1.5 mm. longo, inter lobis laterales 1 mm. lato; columna crassa, brachiis angustissimis; ovariis cylindraceis, glabris, pedicellos aequilongis.

Colombia: Distr. Cauca, ad pag. El Tambo, in silva ad Munchique. Alt. 2700 meters. Nov. 11, 1936. Coll. *K. v. Sneidern No. 1097.* Type in the herbarium of the State Museum of Natural History, Stockholm, Sweden.

SECTION Valvae

Stelis cryptochila Garay, sp. nov. Fig. 2D

Epiphytica, caespitosa, usque ad 20 cm. alta; radicibus fasciculatis, valde flexuosis, filiformibus, glabris; caulibus secundariis erectis, teretibus, in medio basinque vaginatis, vaginis amplis, obliquis, acutis, ad 4 cm. longis; foliis ovato-oblongis, acutis, basin distincte (2–2.5 cm.) petiolatis, cum petiolo usque ad 10 cm. longis, 1.5–2 cm. latis; inflorescentiis ut videtur (an semper ?) singulis, folia superantibus; bracteis ovato-triangularibus, acutis, 2.5–3 mm. longis; floribus succedaneis, bilabiatis, tenuibus, glabris; sepalo postico oblongo, obtuso vel subacuto, 7-nervio, 2.5 mm. longo, 2 mm. lato; sepalis lateralibus usque ad apicem connatis (in natura), valde saccatis, bifidis, 2 mm. longis, 3.5 mm. latis, 8-nerviis; petalis semirotundis, 3-nerviis, apice leviter incrassatis, columnam et labellum enclosis, sepalis multoties brevioribus; labello porrecto, deltoideo-triangulo, obtuso, antice excavato, basin cum callo bipartito ornato; ovariis cylindraceis, pedicellis bracteisque aequilongis.

Ecuador: Prov. Napo-Pastaza, near Tena. Alt. 400 meters. In dense forest. April 2–11, 1935. Coll. *Ynes Mexia No. 7190.* Type in the author's herbarium No. 3368. Isotype in the herbarium of the U.S. National Museum.

Stelis violacea Garay, sp. nov. Fig. 2E

Epiphytica, caespitosa, spithamaea; radicibus filiformibus, fasciculatis, albidis, glabris; caulibus erectis, satis gracillibus, univaginatis, 6 cm. longis; foliis ovatis, obtusis, basin versus conduplicatis et in petiolum augustatis, petiolo incluso ad 7 cm. longis, 2 cm. latis; inflorescentiis 1–3-nis, folia nunc superantibus nunc brevioribus, multifloris; bracteis brevissime tubulosis, ovariis pedicellatis dimidio brevioribus; floribus ringentibus, intus papillosis;

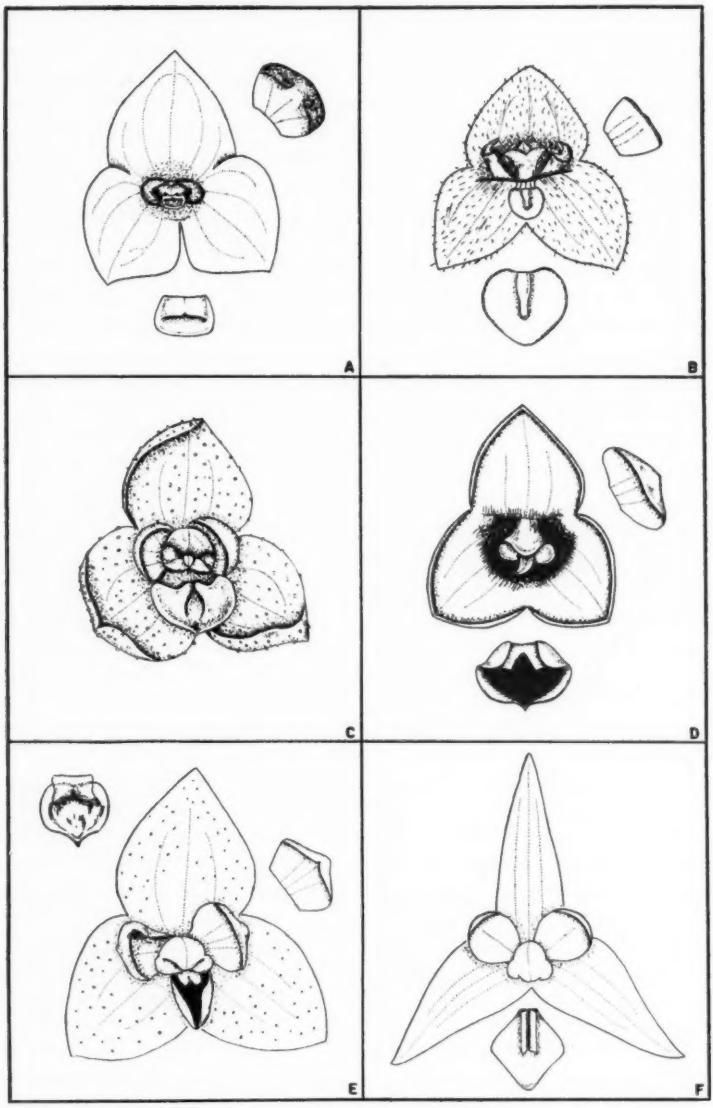

FIG. 1. A. *Stelis convallarioides*, sp. n. Flower. × 4. B. *Stelis Jamesoni* var. *parviflora*, var. n. Flower. × 7. C. *Stelis papillosa*, sp. n. Flower. × 10. D. *Stelis cochlearis*, sp. n. Flower. × 8. E. *Stelis navicularis*, sp. n. Flower. × 8. F. *Stelis rhomboidea*, sp. n. Flower. × 10.

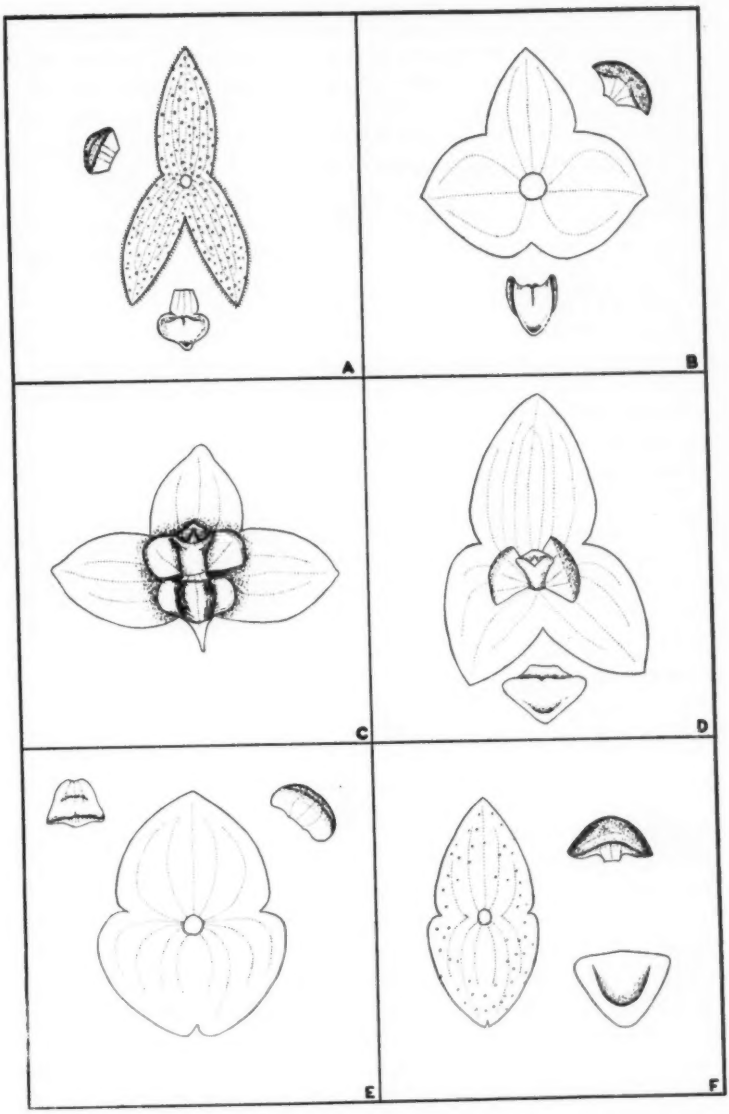

FIG. 2. A. *Stelis sanguinea*, sp. n. Flower parts. × 2. B. *Stelis genychila*, sp. n. Flower parts. × 4. C. *Stelis tenuipetala*, sp. n. Flower. × 10. D. *Stelis cryptochila*, sp. n. Flower. × 14. E. *Stelis violacea*, sp. n. Flower parts. × 4. F. *Stelis ecallosa*, sp. n. Flower parts. × 3.

sepalo postico deltoideo-triangulo, acuto, cum sepali lateralibus valde connato, 5-nervio, 4 mm. longo, 4.5 mm. lato; sepalis lateralibus usque ad $\frac{1}{3}$ partem apicalem connatis, acutis, inter se 8-nerviis, 4 mm. longis, basin 6 mm. latis; petalis transversis, valde carnosis, 1 mm. longis, 1.5 mm latis; labello e cuneato basi dilatato, antice truncato, callo apicali, retuso, disco papuloso incrassato, 1 mm. longo, 1.25 mm. lato; columna humili, alis valde productis; ovariis cylindraceis, pedicellis aequilongis.

Colombia: Distr. Cauca, ad pag. El Tambo, in silva ad Munchique. Alt. 2400 meters. Nov. 11, 1936. Coll. *K. v. Sneidern No. 1096.* Type in the herbarium of the State Museum of Natural History, Stockholm, Sweden.

Stelis ecallosa Garay, sp. nov. Fig. 2F

Epiphytica, usque ad 17 cm. alta; radicibus filiformibus, flexuosis, glabris; caulibus caespitosis, arcuatis cum vaginis binis, brunneis obtectis, 3 cm. longis; foliis ovato-lanceolatis, acutis vel obtusis, basin versus leviter attenuatis, ad 8 cm. longis, 1.2 cm. latis; inflorescentiis singulis, gracillibus, remotifloribus, ad 14 cm. longis; bracteis tubulosis, ovariis pedicellatis brevioribus; floribus satis magnis, bilabiatis; sepalis inter se connatis, intus sparse papillosis; sepalo postico late triangulo, acuto, 5-nervio, 6 mm. longo, 5 mm. lato; sepalis lateralibus usque ad apicem connatis, brevissime bifidis, 6-nerviis, 5 mm. longis et latis; petalis minutissimis, transversis, brevissime unguiculatis, valde carnosis, 1 mm. longis, 1.5 mm. latis; labello carnoso, triangulo, sensim excavato, ecalloso, 1 mm. longo latoque; columna humili, brachiis late rotundatis, valde evolutis; ovariis pedicellos duplo brevioribus.

Ecuador: Prov. Napo-Pastaza; Tena. Oct. 14, 1939. Coll. *Eric Asplund No. 9311.* Type in the herbarium of the State Museum of Natural History, Stockholm, Sweden.

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STUDIES OF SOIL FUNGI

II. A NEW SPECIES OF SPHAEROSPORA WITH A BOTRYTIS-LIKE STAGE[1]

By Roy F. Cain[2] and Nancy A. Hastings[3]

Abstract

A new species, *Sphaerospora minuta* Cain, is described from soil collected under *Pinus banksiana*, Algonquin Park, Ontario. Growth in pure culture is rapid on various media. Conidia are produced in *Botrytis*-like clusters on long, dichotomously branched conidiophores. The conidial stage resembles that of *Patella abundans* (Karsten) Seaver except for the fewer conidia which have a broader attachment to the ampullae. The method of conidial production in both of these species is described in detail and illustrated. Monoconidial cultures of *S. minuta* produce mature apothecia which are globose, light-brown, and enclosed in an excipulum covered with numerous slender brown septate hairs. The excipulum remains intact until the apothecium is nearly mature and then splits irregularly to expose the nearly white hymenium. The ascospores are hyaline, globose, smooth, and each contains a single large refractive oil globule. Consideration is given to the validity of the genus *Sphaerospora* as separate from *Humaria* (*Patella*).

Introduction

This interesting fungus was isolated in pure culture from sandy soil collected in a forest of *Pinus banksiana* at Achray, Algonquin Park, Ontario, on October 5, 1953.

A conidial stage, somewhat resembling a *Botrytis*, is produced in a few days. This is followed in a week's time by mature apothecia. The globose ascospores and hairy excipulum are characteristic of the genus *Sphaerospora* as used by Seaver (10), who includes six species from North America. In three of these the ascospores are smooth-walled, and in the other three they are roughened with spines or warts. In the type species, *Peziza trechispora* Berk. & Br., the ascospore wall has rounded wart-like tubercles. The genus is treated in a similar way by Velenovsky (12). The new species from Ontario has smooth-walled ascospores but differs from those previously described in the size of the apothecia and the excipular hairs. This species is the first *Sphaerospora* for which a conidial stage has been recorded.

Description

Sphaerospora minuta Cain sp. nov. (Figs. 1–57)

Mycelio niveo, cano dein pallide brunneo, ex hyphis hyalinis, tenuiter tunicatis, plerumque 5–8 μ diam., septatis composito. Conidiophoris longis, hyalinis, tenuiter tunicatis, dichotome ramosis, ampullas efformantibus. Conidiis globosis vel subglobosis, 6–14 μ (plerumque 9–10 μ), pallide brunneis,

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levibus. Ascomatibus gregariis, sessilibus, primo globosis, dein subglobosis, versus basim angustatis, 300–1000 μ diam. (plerumque 300–600 μ), extus pallide brunneis, mollibus pilis simplicibus, rectis, rigidis, acutis, numerosis, brunneis, 2–3-septatis, usque 250 μ longis, basi 5–10 μ latis vestitis. Hymenio convexiusculo, albido-subcinereo. Excipulo tenui-membranaceo, parenchymatico, pallide brunneis. Ascis octosporis, cylindraceis, 125–160 × 16–19 μ, apice subtruncatis, versus basim abrupte angustatis, breve stipitatis. Paraphysibus 2.5–5 μ diam., filiformibus hyalinis, septatis, ascos superantibus. Ascosporis monostichis, 13–17 μ diam., globosis, hyalinis vel pallide brunneis, uniguttatis, continuis, levibus.

Conidiophores long, upright, hyaline, thin-walled, 5–8 μ diameter, at first non-septate, branching dichotomously at right angles near apex. Primary branches about 15–30 μ long. Usually one or two additional sets of dichotomous branches with a few septa developing in primary, secondary, and tertiary branches. Ultimate end of each branch producing subglobose, conidium-like ampullae in branching chains and not separated by septa. Conidia two to four on each terminal ampulla, forming a terminal crown or one to three laterally on subterminal ampulla, readily deciduous, separating at septa or just below leaving a distinct scar measuring 3–4 μ (rarely up to 7 μ) in diameter; globose or subglobose, somewhat variable in size, 6–14 μ in diameter (mostly 9–10 μ), very light-brown, smooth, without refractive or granular content, with a rather thick wall, 0.5–1.5 μ in thickness.

Apothecia developing from coiled ascogonia on aerial hyphae, globose when young and completely enclosed in the excipulum, at maturity subglobose, 300–1000 μ in diameter (mostly 300–600 μ), superficial, sessile, contracted to a narrow base, externally light-brown, soft and fleshy throughout. Hymenium nearly white, usually remaining convex, rarely becoming nearly flat, extending beyond the irregular margin of the excipulum. Excipulum cup-shaped, thin, 30–50 μ in thickness, three to four cells in section, light-brown, covered with brown projecting hairs, splitting irregularly to expose hymenium. Cells of excipulum swollen, thin-walled, subglobose or usually somewhat elongate, in outer layer up to 50 μ long (mostly 15–25 μ), very light-brown, smaller, and more elongate in inner layer where they merge with paraphyses. Hypothecium white, thin, consisting of small hyaline cells. Hairs arising from outer cells of excipulum, numerous, straight, unbranched (rarely once branched near base), rigid, up to 250 μ long (mostly about 100–200 μ), 5–10 μ wide near base, tapering gradually to apex which may be sharp-pointed or blunt, with two to three (usually two) septa near base, wall 1.0–1.5 μ thick with scattered, dome-shaped projecting tubercles on upper part or nearly smooth, brown, nearly hyaline toward base, basal cells somewhat swollen.

Asci eight-spored, cylindrical, tapering slightly above spores to a broadly rounded apex, 125–160 × 16–19 μ, without visible thickening in wall, rarely with one large refractive globule in apical part, rather abruptly contracted below into a short stipe, with only a short clear space below the ascospores, developing from a crozier at base; operculum of ascus not seen.

Paraphyses 2.5–5 μ in diameter (mostly 3 μ), filiform, branching near base, hyaline, septate, upper ends nearly straight with uniform diameter, broadly rounded at apex, intermingled with asci and projecting above them.

Ascospores uniseriate, readily becoming biseriate in crushed water mount, 13–17 μ, globose, hyaline, with single refractive oil globule measuring 7–10 μ in diameter, and usually a few small globules. Ascospore wall 1 μ in thickness, smooth in appearance except when stained with iron-alum haematoxylin which stains scattered surface granules dark, giving wall a slightly roughened appearance.

Isolated in culture from soil collected under *Pinus banksiana*, Achray, Algonquin Park, Ontario, Canada, Oct. 5, 1953. *Type* TRTC 30102.

Cultural Characteristics of *Sphaerospora minuta*

Colonies develop very rapidly and produce first a conidial and then an ascocarpic stage on most of the several types of media that have been used. Both conidia and apothecia as well as a fairly extensive growth of mycelium were produced on 1% malt, 1% malt + peptone, and Leonian's + 0.1% yeast extract. On Czapek's agar, the mycelium was less extensive, conidia were scarce, and the apothecia were small and failed to mature properly. On Jeffrey's agar the colony was very restricted owing to the inhibiting action of rose bengal. Cultures were maintained by frequent transfers for about a year and during that time showed no decrease in their ability to produce apothecia.

Cultures were obtained from single conidia. These grew just like the colonies from mass transfers and produced mature apothecia with normal ascospores. Monoascospore cultures were attempted and all failed to germinate even after heat treatment.

At room temperature, Petri dishes with malt agar are completely covered in two days with a very thin, white, glistening, loose, surface layer of mycelium, extensive areas of which, up to one centimeter in thickness, are of white cottony conidiophores. With maturity of conidia the culture darkens to a light ochraceous buff ("Cartridge Buff" to "Chamois", Ridgway). This color is maintained for a week or two or it may become slightly darker as the apothecia mature because of the darker hairs on the excipulum. On the third day there is considerably more aerial growth forming cottony areas with more conidiophores. The submerged hyphae are very irregular with many swollen portions, hyaline, thin-walled, with numerous oil globules, branching and anastomosing frequently. The surface hyphae are hyaline, much-branched, rarely anastomosing, with short, irregular, somewhat swollen cells 5–18 μ in diameter (Fig. 56). The aerial hyphae begin to develop on the third day as branches from the surface mycelium and are more regular. Some of these are hair-like, 4–7 μ wide at the base, tapering gradually to 1.5 μ at the apex, long, slender, straight, with rigid wall but not thickened, minutely but distinctly roughened, light yellowish-brown, with a few indistinct septa, giving the culture a light ochraceous buff appearance. Cultures

exposed to summer temperature of about 85° F. have mature conidia in about 24 hr. At more moderate temperatures this requires two or three days. Ascogonia appear about the third day and apothecia mature when the culture is about a week to 10 days old.

Conidial Stage of *Sphaerospora minuta* (Figs. 18–47)

The rather long conidiophores form a loose layer up to a centimeter in thickness. They are upright, hyaline, thin-walled, rather broad (5–8 μ), branching dichotomously at right angles near the apex (Fig. 18). The primary branches are about 15–30 μ long. Usually there are one or two more sets of dichotomous branches with a few septa developing in the primary, secondary, and tertiary branches (Figs. 19–32). The branching at any level may be somewhat irregular (Fig. 20). The ultimate end of each branch produces a swelling which becomes subglobose and conidium-like. This proliferates at the end into a second swelling similar to the first. Each swelling is considered as an ampulla (Figs. 21–23). An ampulla frequently forms two distal ampullae so that a rather irregular cluster or head is formed (Fig. 25). The terminal ampullae produce two to four conidia in the form of a crown around the outer part (Figs. 35–45). The ampullae at the end of each branch are not separated by septa. The subterminal ampullae may produce one to three conidia laterally (Figs. 41, 44, 45) but more frequently develop ampullae only (Figs. 28, 37). In the early stage the conidia are subglobose, without a septum at the base, and resemble the developing ampullae (Figs. 26, 30, 36, 38, 39, 43, 46). But as the conidium matures it is separated from the ampulla by a septum, its attachment being a rather wide base which usually measures 3–4 μ but may be up to 7 μ (Figs. 40, 41, 44, 45, 47). The septum usually forms at the constriction just below the conidum, rarely with a short neck between septum and globose part. The conidia are readily deciduous separating at the septum or just below to leave a distinct scar at the point of attachment (Fig. 33). They are globose or subglobose, somewhat variable in size ranging from 6–14 μ in diameter, but mostly 9–10 μ, light-brown, smooth, without refractive or granular content, with a rather thick wall which is 0.5–1.5 μ in thickness.

Conidial Stage of *Patella abundans* (Figs. 58–62)

In general appearance the cultures of *S. minuta* are indistinguishable from those of *Patella abundans* (Karsten) Seaver. Apothecia of this fungus appeared on moist felt paper in a moist chamber in our laboratory during March, 1954. For the purpose of comparison a pure culture was obtained. Our cultures of *Patella* developed a conidial stage of the *Botrytis* type as described by B. O. Dodge (1). The conidiophores are upright, about a millimeter in length, usually unbranched below, and developed from the scanty

aerial or surface mycelium. The conidiophore (Fig. 58) begins to branch dichotomously near the upper end so that the primary branches are about 100–200 μ long, the secondary a little shorter, 50–65 μ, and the tertiary only 20–30 μ long. The branches are about the same width as the conidiophore (7–10 μ). The conidiophore continues to branch dichotomously but with shorter branches so that compact heads of conidia are produced. The extent of branching depends on the richness of the medium; for example, on malt agar about seven dichotomies develop. The end of each branch is swollen to form a globose ampulla 10–15 μ in diameter (Fig. 61). Each ampulla produces simultaneously a series of about 10–15 denticles, 2–3 μ long and 1 μ wide (Figs. 59, 60, 61). Each denticle is cylindrical at first and then produces a globose swelling at the tip which enlarges to form the globose conidium. An indistinct septum forms in the mid-portion of the persistent denticle. The conidium breaks away below the septum, leaving part of the denticle on the conidium and part on the ampulla, which then collapses along with the branches of the conidiophore. The conidia are globose, slightly depressed opposite the apiculus, smooth, with a fairly thin wall, faintly brown in color (ochraceous in mass) (Fig. 63). All conidia in a head are produced simultaneously. A description and illustration of the conidial stage of *P. abundans* was published by Kevorkian (5).

In spite of the numerous small denticles the method of conidial production here is not fundamentally different from that typical of the "blown-out ends" of the apices of conidiophores (3). In *P. abundans* each end becomes swollen (to form the ampulla) and then produces numerous minute branches (the denticles). The conidia are the "blown-out ends" of these and are cut off by a normal septum. This is also precisely what happens in the so-called oedocephaloid head in the Imperfect genus *Oedocephalum*, where a single swollen end of the conidiophore develops numerous denticles produced simultaneously each with a conidium at the apex. The *Botrytis* stage of *Patella abundans* differs from *Oedocephalum* only in having the upper part of the conidiophore branched to produce a series of small oedocephaloid heads (or ampullae).

Interpretation and Discussion of Conidial Stage

The conidia of *Sphaerospora minuta* may be considered to be the "blown-out ends" (3) or swollen apices of the conidiophores, which in this case are branched and swollen, and each produces one to four short branches (the denticles) each with a conidium at the end. These are produced simultaneously. The method of production is essentially like that of conidial *Patella abundans* but differs as follows: the ampullae develop in short branching chains, each ampulla produces 0 to 4 conidia instead of 10–15, the denticles are shorter and much broader. In each species the denticles are interpreted as branches that develop simultaneously from the swollen end of the conidiophore (the ampulla). It is probable that the denticles of many other species are to be interpreted similarly, for example, species of *Oidium* (*Rhinotrichum*).

In all these cases, more or less minute branches are produced simultaneously in more or less large numbers on the parent cell and each swells out at the apex, i.e. is "blown-out", to form a conidium which becomes separated by an internal septum formed in the constriction. The parent cell may be in the aerial hyphae or on distinct conidiophores. In essential features this type of conidium is not very different from the terminal chlamydospore. It is entirely vegetative in origin and quite different from the conidium produced on a phialide which is interpreted as spermatial in derivation.

In spite of the distinct differences in the method of conidial production between *Sphaerospora minuta* and *Patella abundans*, when the many similarities are taken into account, a close family relationship is indicated. However, the mere fact of conidial production in botryose heads does not always mean a close relationship, since *Botryotinia* and *Seaverinia* of the Sclerotiniaceae also have an imperfect stage of the *Botrytis*-type. In both of these fungi there are many more significant differences from *Patella abundans* and *Sphaerospora minuta* in details of conidial and conidiophore structure. The botryose head of conidial production has apparently evolved independently in several evolutionary lines. The same can be said for the oedocephaloid type found in various genera including *Aleuria*, *Pustularia*, *Plicaria*, *Peziza*, and *Pyronema* among the Operculate Discomycetes (11, 13) and *Fomes annosus* (Fr.) Cooke, *Stereum sulcatum* Burt, *Corticium furfuraceum* Bres., and others belonging to the Basidiomycetes (7).

Apothecial Development in *Sphaerospora minuta* (Figs. 48–53)

The ascogonia appear as side branches on the aerial hyphae when the culture is two to three days old. These branches are broader than the hyphae and measure about 10 μ in diameter (Fig. 53). Sometimes the ascogonium becomes spiraled rather regularly with up to six coils, the lower one being much broader (Fig. 52). More usually it becomes twisted in an irregular coil (Figs. 48, 50, 51). Transverse septa are formed at irregular intervals. Branches develop from the basal part of the coil. These rebranch and grow upward to enclose the irregularly coiled ascogonium (Fig. 49). In one case a distinct fusion was observed between a coil of the ascogonium and what may have been a branch on the outside (Fig. 50). The ascogonial coils and young apothecia usually remain quite distinct from adjacent aerial hyphae. The branches enclosing the ascogonium develop shorter cells to form a pseudoparenchyma which develops into the excipulum which continues to enclose the layer of asci until maturity of the apothecium.

The apothecia remain nearly globose and completely enclosed in the excipulum until mature and almost full size (Fig. 2). When the culture is about 7 to 10 days old, the excipulum splits irregularly to expose the nearly white convex hymenium (Figs. 1, 3). The excipular hairs become distinctly brown (Figs. 4–6, 13) and form a distinct fringe around the outside of the apothecium, while the excipulum itself remains light brown.

Asci mature at a fairly early stage in the development of the apothecium and while the excipulum is still unbroken (Figs. 8–12, 14, 15, 17). They are formed with a crozier at the base. Discharge of the ascospores from the asci has not been observed and no open opercula have ever been formed. When crushed in a water mount, the cylindrical asci swell out and burst in the mid-portion. They are fairly persistent in the fresh apothecia but tend to break down in the dried material. Such observations lead almost inevitably to the conclusion that we have here an apothecial operculate which has evolved almost to the point of production of cleistothecia with indehiscent asci.

The ascospores each contain a characteristic, large oil globule and the wall becomes rather thick but remains smooth (Fig. 34). At maturity they may appear very light brown in color. In one case a giant biguttulate ascospore was observed replacing two spores in the ascus.

When the dried apothecia were mounted in Melzer's solution there was no iodine reaction observed in the excipulum, asci, paraphyses, or ascospores.

Classification

The fungus is characteristic of species belonging to the genus *Sphaerospora*, which differ from species of *Patella* in having globose instead of ellipsoid ascospores. The name *Lachnea* has been used instead of *Patella* but is untenable because that name was used previously for a genus of flowering plants. The genus *Barlaea* also has globose ascospores but lacks the hairs which characterize the apothecia of *Sphaerospora*. The valid generic name for *Patella* appears to be *Humaria* as pointed out by Kanouse (4).

Sphaerospora was raised to generic rank by Saccardo (9) who had previously used the name as a section of the genus *Peziza* (8). It differs from *Patella* in having globose ascospores and from *Barlaea* in having hairs on the apothecia. The type of the genus is *S. trechispora* (Berk. & Br.) Sacc., the first species included by Saccardo (9). It is one of the more common and better known species.

The following species of *Sphaerospora* have been described: *Peyronemella araneosa* Sacc. (hairy but hairs not bristly), *Sphaerospora asperior* Nyl. (near *S. trechispora*), *S. barlae* Boud., *S. citrina* Mass. & Crossl., *S. confusa* (Cooke) Boud. (Seaver uses *S. brunnea* (Alb. & Schw.) Massee), *S. confusa* var. *ochracea* Rehm, *S. diaboli* Vel., *S. flavo-virens* (Fuck.) Symb. (includes *S. durandi* Rehm), *S. hinnulea* (Berk. & Br.) Massee (includes *S. scutelloides* Ellis), *S. lancifera* Hazsl., *S. lasioboloides* March., *Pyronemella limnophila* Beck, *S. minor* Vel., *S. monilifera* (Berk. & Curt.) Seaver (*Pyronemella*), *S. patagonica* Rehm, *S. perplexa* Seaver, *S. porphyra* Berk. & Curt., *S. sordida* Vel., *S. staritzii* P. Henn., *S. strasseri* Bres., *S. trechispora* (Berk. & Br.) Sacc., *S. verruculosa* Berk. & Br.

These all differ in some essential feature such as size and color of apothecia, size of ascospores, or marking of ascospore wall. Conidia have been described for none of these.

This makes a total of 21 species described in the literature including two species of *Pyronemella* (with less bristly hairs) not yet transferred to *Sphaerospora*. There are a few additional names which have been reduced to synonomy.

Seaver (10) lists six species known from North America. Velenovsky (12) gives six species from Czechoslovakia. Of these, three are new species described by Velenovsky and two are also known from North America. Grelet (2) gives four species from France, but includes these in the genus *Ciliaria*.

Most of the species of *Sphaerospora* have been described as occurring on soil, as does our new species, and most of them are larger than this species. Some have more brilliantly colored apothecia (such as red), and in some the ascospores are ornamented with sharp crowded spines.

LeGal (6) and Grelet (2) use the genus name *Ciliaria* to include species of *Sphaerospora* with globose ascospores as well as a number of species of *Patella* with ellipsoid ascospores. However, there seem to be some advantages in keeping the species with globose ascospores in a separate genus, for which *Sphaerospora* appears to be the valid name. The conidial stage of the new species of *Sphaerospora* bears some resemblance to that of *Patella abundans* (*Ciliaria abundans*), but, as noted above, shows considerable difference in the method of conidial production. This difference seems to be sufficient to warrant keeping *Sphaerospora* as a separate genus, at least until more information is available concerning the conidial stage of the other species.

For this new species we propose the name *Sphaerospora minuta* on account of the small apothecia. It is possible that in culture the apothecia are smaller than they would otherwise be under natural conditions.

In addition to the small apothecia, the species is distinguished by the light-colored apothecia, the small, smooth ascospores and the *Botrytis*-like conidial stage.

Sphaerospora brunnea (Alb. & Schw.) Massee is most likely to be confused with *S. minuta* because of the very similar asci and ascospores. A collection of the former was found on burnt soil in a beech–maple–hemlock woods at Nashville, York Co., Ontario, on October 1, 1955 (TRTC 31348). The apothecia are much larger being 5–6 mm. in diameter, more distinctly discoid, and darker in color, especially the hymenium. The hairs are lighter in color and of a different shape being shorter and more triangular as figured by Boudier in Icon. Mycol. Pl. 379.

It might be argued that the method of conidial production in the *Sphaerospora* is sufficiently different to warrant the erection of a new imperfect genus distinct from *Botrytis*. But there is nothing to be gained by the use of a large number of genera of the Fungi Imperfecti based on small differences. It must be recognized that *Botrytis* as usually used comprises a large number of morphologically different forms of conidial production all having botryose clusters of conidia, and representing several genetically distinct fungi in the ascospore stage. Since the sorting out of *Botrytis* spp. into various genera

would be difficult and of little value, it is much more convenient to continue to use *Botrytis* as a form genus to include species in which the conidia are produced in multiple numbers simultaneously on the swollen ends (ampullae) of branching stout conidiophores to form botryose clusters.

Certainly, the conidial stage of the new *Sphaerospora* differs considerably from all other known types of Fungi Imperfecti but especially since the perfect stage is known there seems to be no need to provide a new generic name for the Imperfect stage. This can best be referred to as the *Botrytis* stage.

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EXPLANATION OF FIGURES

FIGS. 1–57. *Sphaerospora minuta.*

FIG. 1. Apothecium, side view. × 50. FIG. 2. Unopened apothecium, longitudinal section. × 50. FIG. 3. Open apothecium, longitudinal section. × 100. FIG. 4. Cells of excipulum and base of apothecial hairs. × 600. FIG. 5. Single hair of apothecium, broken in middle, showing a branch at base. × 1140. FIG. 6. Apex of hair showing blisters in wall. × 1140. FIG. 7. Cells of excipulum. × 1140. FIG. 8. Two immature asci with croziers at base. × 1140. FIG. 9. Young ascus showing a globule in apex. × 500. FIG. 10. Mature ascus. × 500. FIG. 11. Young ascus with refractive globules, before spores are cut out. × 500. FIG. 12. Very young asci developing from croziers. × 500. FIG. 13. Hair from excipulum. × 500. FIG. 14. Mature ascus. × 500. FIG. 15. Ascus with immature spores. × 500. FIG. 16. Branching paraphyses. × 500. FIG. 17. Mature ascus. × 500.

FIG. 18. Apex of young conidiophore showing first dichotomous branching. × 1140. FIG. 19. Apex of young conidiophore showing second dichotomous branching. × 1140. FIG. 20. One of the primary branches of young conidiophore showing three instead of the normal two secondary branches and six ampullae beginning to form on the tertiary branches. × 1140. FIG. 21. Apex of young secondary branch of conidiophore showing ampullae producing spores. × 1140. FIGS. 22–23. Secondary branch of conidiophore with tertiary branches and branching ampullae. Conidia not yet formed. × 1140. FIG. 24. Primary branch of conidiophore with two secondary branches, tertiary

branches, and ampullae, some of which are branching. Conidia not yet formed. × 1140. FIG. 25. End of conidiophore with mostly dichotomous branching to form ampullae. Conidia not yet formed. × 1140. FIG. 26. Apex of primary branch of conidiophore with ampullae and young conidia in various stages of development. The single globose structure near base at the left is a conidium. × 1140. FIG. 27. Primary branch of conidiophore with secondary and tertiary branches and mature conidia which obscure the ampullae. × 1140.

FIG. 28. Two terminal ampullae each produced at apex of an ampulla with terminal conidia nearly mature. Secondary and tertiary branches are shown. × 1140. FIG. 29. Two terminal ampullae with terminal crown of conidia and three subterminal ampullae with four lateral conidia. × 1140. FIG. 30. Conidiophore with primary, secondary, and tertiary branches with branching chains of ampullae producing terminal and a few lateral conidia in various stages of development. × 1140. FIG. 31. Primary branch of conidiophore with secondary and tertiary branches. The upper tertiary branch has a subterminal ampulla with a lateral conidium and a terminal ampulla with three conidia. The lower branch is similar except for the extra terminal ampulla with two conidia. All conidia are nearly mature. × 1140. FIG. 32. Primary branch of conidiophore with two secondary branches, tertiary branches, branching ampullae, and nearly mature conidia. × 1140. (Note: FIGS. 20, 21, 28, 30, and 32 are from a 24-hr. culture.)

FIG. 33. Conidia with basal scar. × 1140. FIG. 34. Ascospores showing thick wall and oil globules. × 1140. FIG. 35. End view of ampulla with crown of three conidia. × 1140. FIG. 36. Terminal ampulla with two conidia. × 1140. FIG. 37. Subterminal ampulla and terminal ampulla starting to produce a conidium. × 1140. FIG. 38. Subterminal ampulla and terminal ampulla with one small and one large conidium. × 1140. FIG. 39. Terminal ampulla with two conidia of different sizes. × 1140. FIG. 40. Terminal ampulla with two conidia (one separated by septum). × 1140. FIG. 41. Terminal ampulla with two conidia and subterminal ampulla with one lateral conidium. × 1140. FIG. 42. End view of ampulla with crown of four conidia. × 1140. FIG. 43. Terminal ampulla with two conidia. × 1140. FIG. 44. Terminal ampulla with two conidia and subterminal ampulla with two conidia. × 1140. FIG. 45. Ampullae with conidia, some with separating septum. × 1140. FIG. 46. Terminal ampulla and single conidium. × 1140. FIG. 47. Ampullae, on two tertiary branches, and terminal conidia. × 1140. FIG. 48. Hypha with a branch bearing two ascogonial coils one developing excipular hyphae from basal cell of ascogonium. × 1140. FIG. 49. Hyphae investing ascogonium and producing excipulum. × 770.

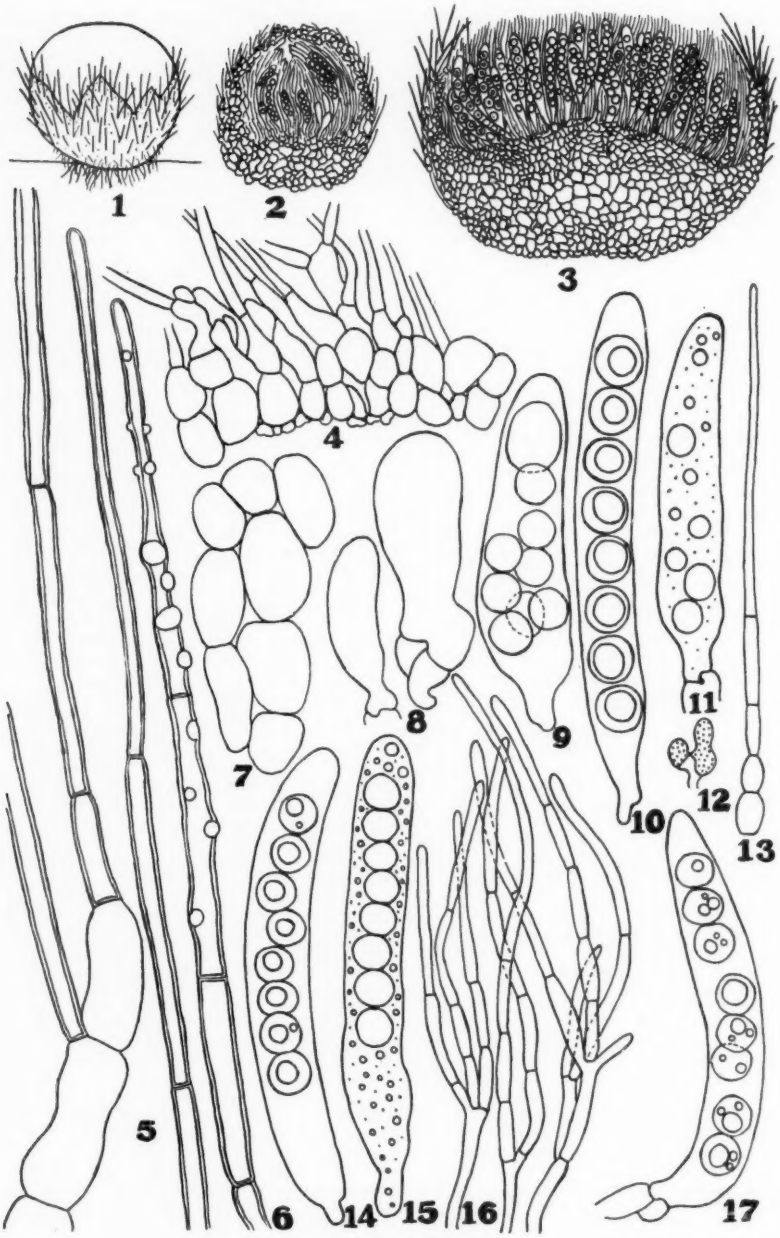
FIG. 50. Irregularly spiraled and twisted ascogonium showing a fusion between upper part and a narrow filament which may be a branch from base. × 1140. FIG. 51. Irregularly spiraled ascogonium from a three-day-old culture. × 1140. FIG. 52. Regularly spiraled ascogonium with six coils showing a septum and basal branches. × 1140. FIG. 53. Branch from a hypha developing into an ascogonium. × 1140. FIG. 54. Basal part of hair from apothecium showing a branch at base. × 1140. FIG. 55. Hair from apothecium with basal cells. × 770. FIG. 56. Hyaline smooth hyphae with irregularly swollen cells from surface of agar. × 770. FIG. 57. Upper part of ascus with refractive globule in apex and eight ascopores. × 1140.

FIGS. 58–62. *Patella abundans.*

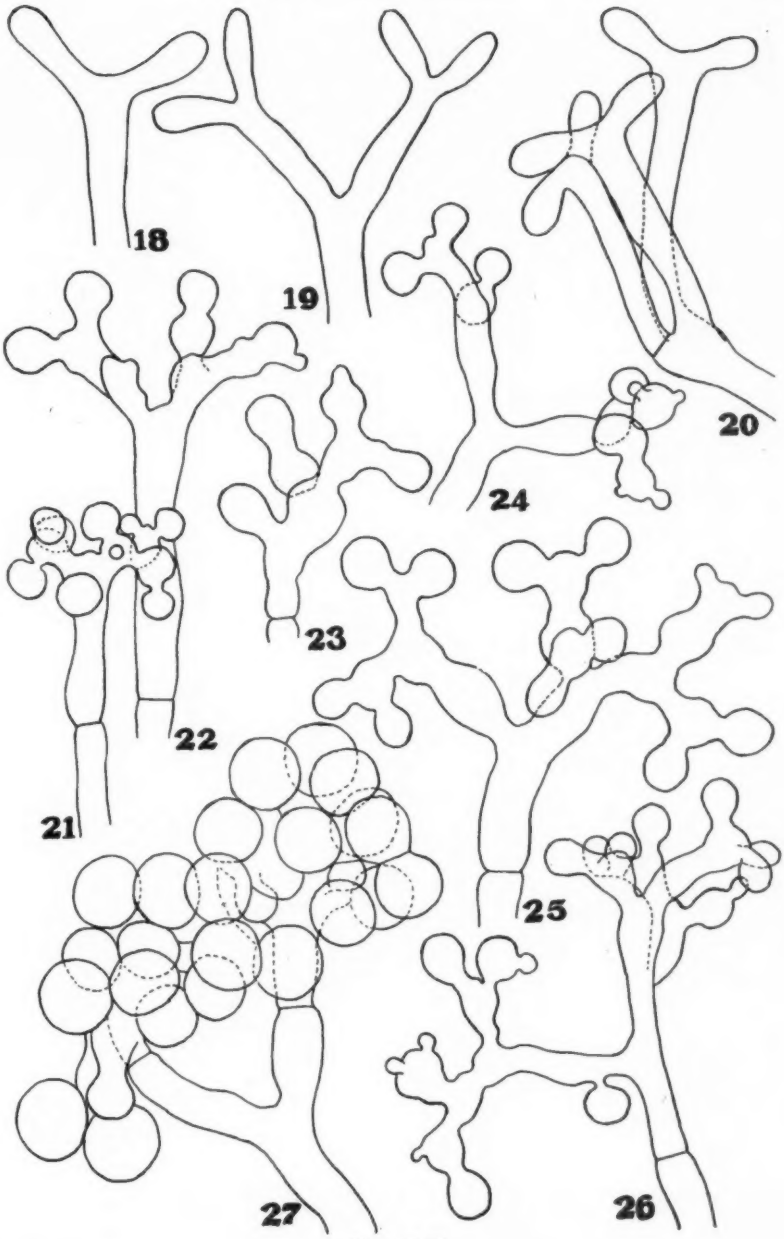
FIG. 58. One conidiophore (broken at B-B, C-C, and D-D) showing primary branching (above C), and one secondary branching (above D), and one tertiary branching, with the two tertiary branches. The one at left has only three heads of four ampullae each. The one at right is normal with four heads of four ampullae each as the result of dichotomies at four levels. × 770.

FIG. 59. Pair of ampullae with conidia beginning to develop. × 1140. FIG. 60. One of the four branches of a conidiophore with eight pairs of ampullae each with conidia beginning to develop. × 1140. FIG. 61. One of eight heads on a conidiophore, there being three dichotomous branchings below this. The fourth dichotomy is shown in the center. Each of the two branches has a fifth dichotomy. There are four dichotomies at the sixth level and eight dichotomies at the seventh, only one of which is shown at the upper left. Sixteen ampullae are just beginning to develop but seven of these are hidden below the upper ones. × 770. FIG. 62. A pair of ampullae produced by the seventh dichotomy of the branch, showing denticles from which the conidia have fallen. × 770.

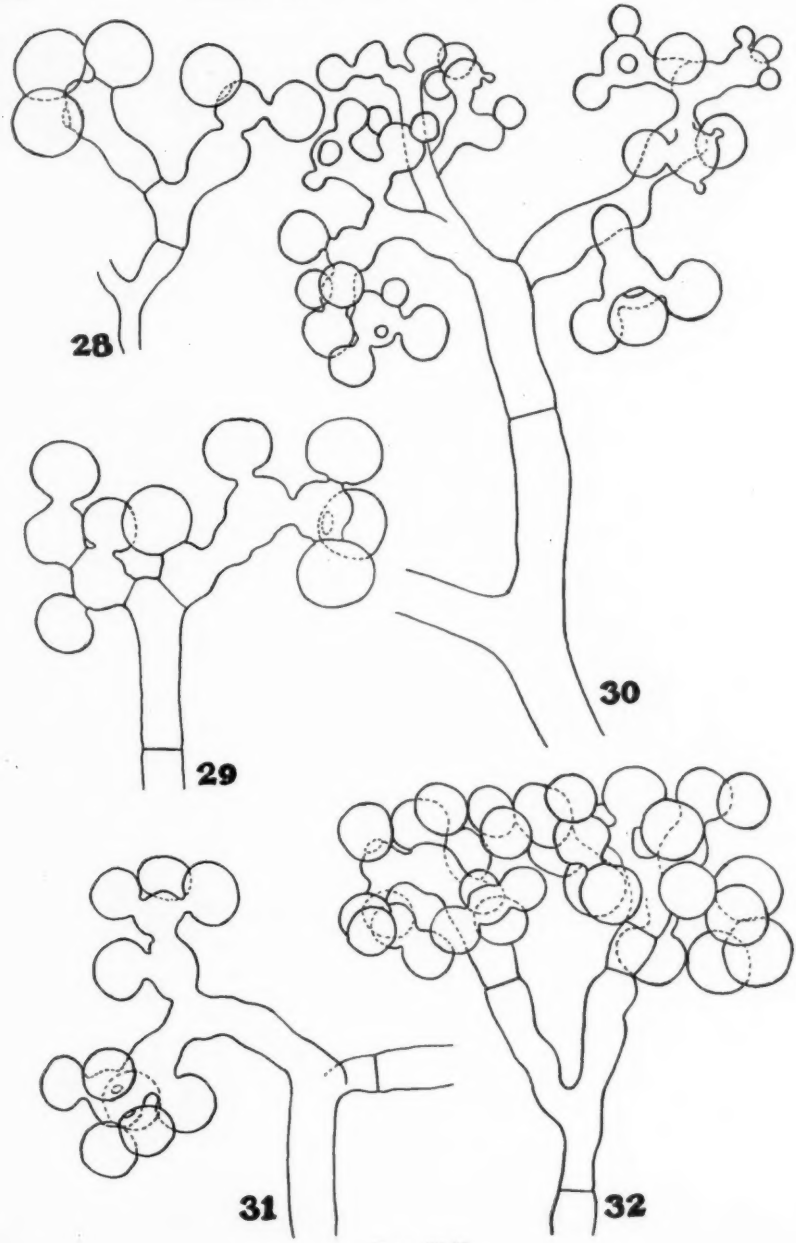
NOTE: FIGS. 1–62 follow.



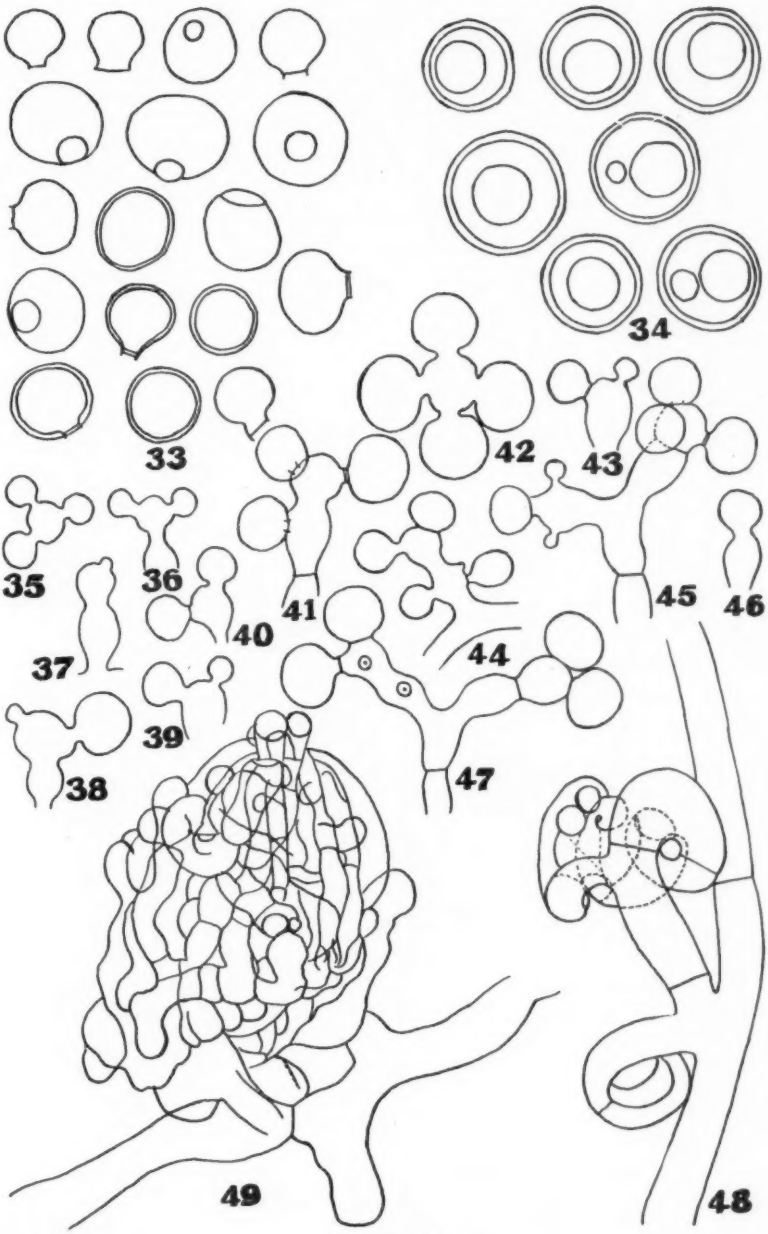
FIGS. 1-17



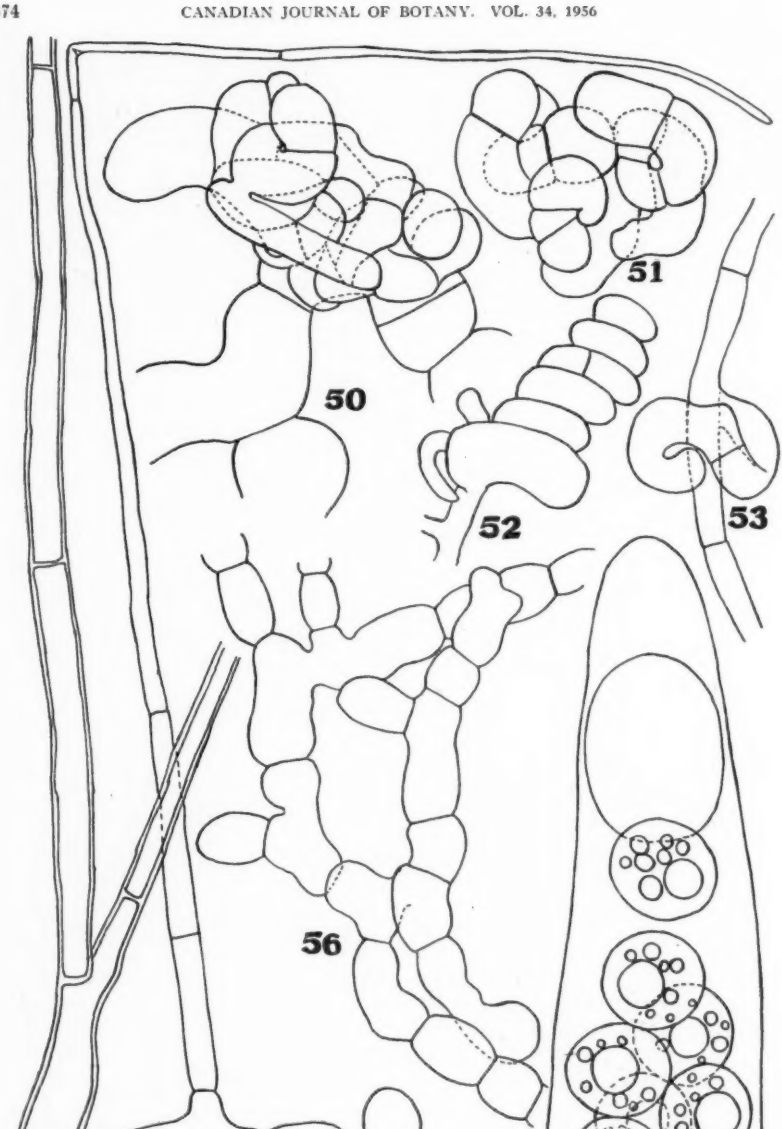
FIGS 18-27



FIGS. 28-32



FIGS. 33-49



FIGS. 50-57

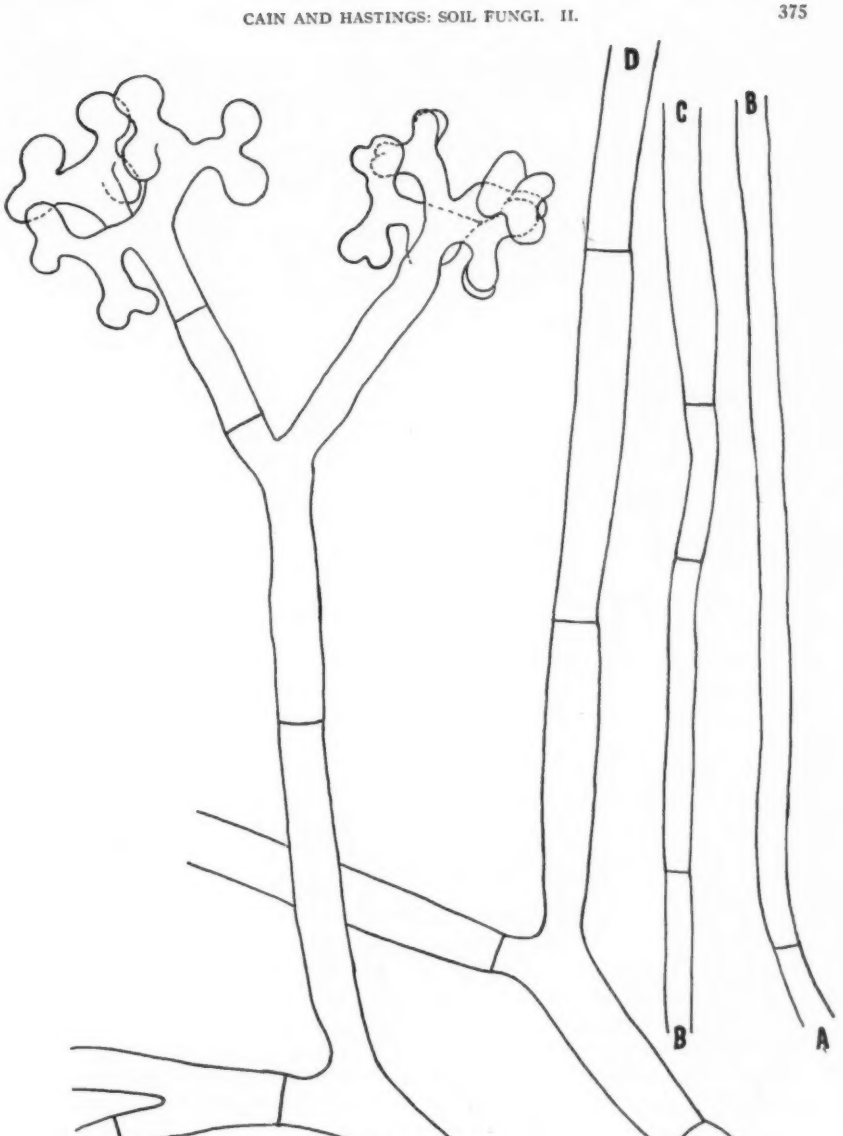
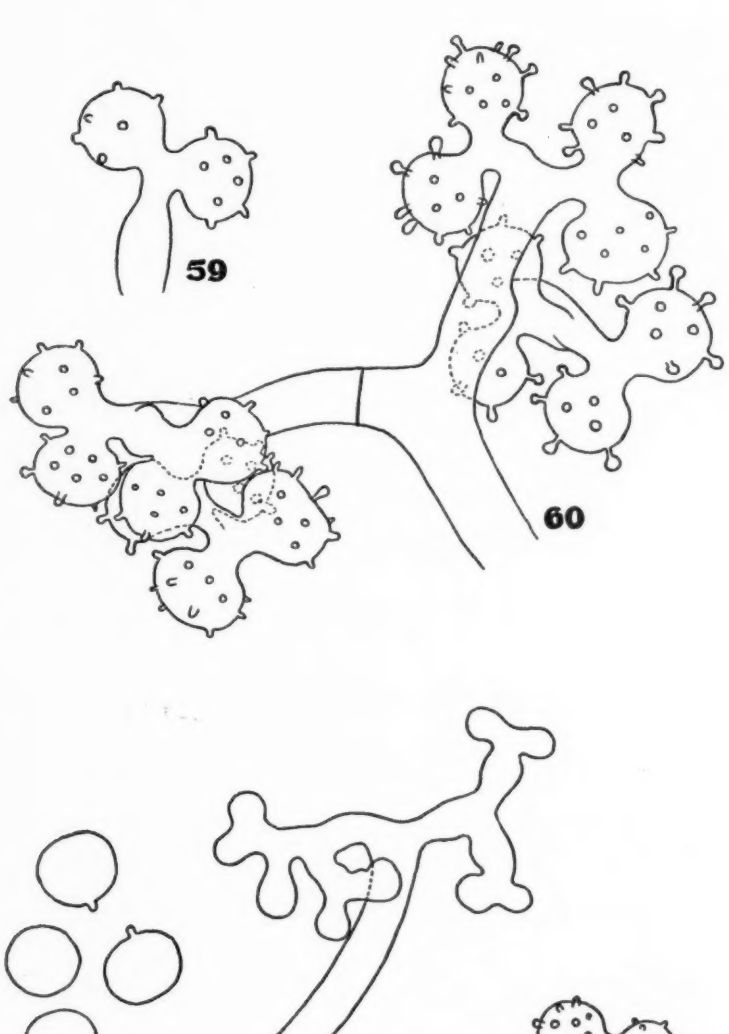


FIG. 58



FIGS. 59-62

PHOTOPERIODIC RESPONSE IN GERMINATION OF SEED OF CERTAIN TREES[1]

By O. Vaartaja[2]

Abstract

Unstratified seeds of *Betula pubescens* Ehr. and *B. verrucosa* Ehr. exhibited low or no germination at temperatures from 10° to 20° C. unless exposed to continuous illumination or illumination interrupted only by a short daily dark period. Germination was high even under a short primary light period if the long dark period was divided into two short ones by an additional daily light period. Besides these true photoperiodic responses, germination was slightly correlated to illumination intensity and to the amount of light. Germination was somewhat reduced when the illumination intensity was either decreased or increased from an optimum. At temperatures of 20° to 25° C., germination was fair, even under a short daily light period. At still higher temperatures no light was needed for maximum or nearly maximum germination. Seeds of three other tree species also germinated better if exposed to long light periods, but the differences were small or not significant statistically. In most of the experiments the effect of temperature was separated from that of light by using running water as the germination medium. The antibiotic Rimocidin as well as various other chemicals and ultrasonic treatments failed to affect the germination of *B. verrucosa*.

Introduction

Visible radiation promotes germination of some seeds (4, 5, 9, 10, 13). Germination in many tree species is promoted if the seeds are exposed to light (1, 2, 7, 13, 14). The minimum intensity of illumination required to promote germination is as low as that of moonlight for certain tobacco varieties (9). In studies by Haack (7), germination of *Picea abies* (L.) Karst. was increased by an illumination of 1/13 lux (1/130 ft-c.) and that of *Pinus sylvestris* L. by illumination of 63 or 163 lux (6.3 or 16.3 ft-c.). The minimum duration required to increase germination varies greatly. For certain tobacco varieties this is only 0.01 sec. if the light intensity is high, and 15 min. if the intensity is low (9). Germination of fresh seed of *Pseudotsuga taxifolia* (Poir.) Britton has been increased by an exposure of a few minutes (1). Certain lettuce varieties also require an exposure of a few minutes (4) and *Epilobium* spp. about 24 hr. (8, 12).

In a study on seed mortality under various conditions, Vaartaja (16) found that seed of several tree species germinated better under long (24 hr.) daily light periods than under short (10 hr.) ones. The stimulating effect of long photoperiods was pronounced with the seed of *Betula pubescens* Ehr. and *B. verrucosa* Ehr., especially at temperatures below 20° C. Since no literature was found concerning photoperiodic responses in seed germination, additional studies were undertaken, mainly with these two species.

Meanwhile other reports have been published about photoperiodic responses of seed. According to the studies of Isikawa and Shimogawara (8) certain

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species of various genera, e.g. *Hypericum* and *Spiraea*, require long photoperiods for high germination. Black and Wareing (3) obtained results similar to those of Vaartaja (16) concerning germination of *B. pubescens* seed. They demonstrated the requirement of long photoperiods for high germination of this species and the dependence of this on temperature. In addition they showed that the length of the daily dark period plays a more determinative role than does the light period in this photoperiodic response, as it does in the flowering responses in plants. Furthermore, these workers found that light is not needed for high germination if the seed has been stratified. Redmond and Robinson (13) made the very interesting discovery that seed coats of *B. lutea* Michx. f. contain a water soluble agent which inhibits germination and which loses this effect when exposed to light.

In the present study, a method was developed to separate the effects of radiation and heat. The seeds were germinated in running water which immediately carried away the heat produced by radiation. This appeared desirable because thermocouple measurements (15) showed that temperature within a dark pine seed exposed to direct sunlight may reach 69° C. while the air temperature (at the height of 6 ft.) is about 25° C. This extreme difference will not occur if the germination medium is moist. Still, seeds in closed Petri dishes, as is the practice in routine tests, tend to get considerably warmer than surroundings if they are exposed to strong radiation. Utilizing running water also precluded the possible diffusion from seeds to the medium of substances that might inhibit germination.

Methods

The general method was to test germination of seed under various temperatures and illumination conditions. In most tests the days were artificially divided into one light and one dark period corresponding to days and nights. If not otherwise specified the length of the photoperiod refers to the daily light period. Various light conditions were arranged utilizing direct or indirect sunlight, fluorescent tubes of daylight type, automatic time switches, and enclosed cabinets.

The seeds tested were of 18 origins in Finland. Since the tests were carried on for several years, mostly from 1951 to 1954, seed lots of varying qualities were used. Therefore the results of the 10 different experiments reported here are not exactly comparable. The work was done at various research institutes and the details of the methods varied according to the facilities available. Different germination media were used as indicated later.

Germination was expressed as percentages of the number of full seeds used. Before the data were analyzed, the germination percentages were transformed into angles (= arc sin $\sqrt{\text{percentage}}$).

Birch seed was first examined in transmitted light, and only well-filled seeds were used. It was found that poor germination occurred in seeds which were only half or less filled.

Germination in Rooms in Winter (Expt. 1)

Seeds were germinated on sterilized quartz sand in pots in two rooms near windows. The seeds were exposed to the following daily illumination.

(*a*) 24 hr. Continuous fluorescent light of 12 ft-c. in addition to 7 hr. indirect sunlight with maximum intensities of 20 to 50 ft-c.

(*b*) 8 hr. (approx.). Natural photoperiod in February in Helsinki. Indirect sunlight with maximum intensity of 20 to 50 ft-c.

(*c*) 5 hr. Fluorescent light of 12 ft-c. in addition to indirect sunlight (5 hr.) with maximum intensities of 30 to 50 ft-c.

(*d*) Darkness continuously.

The radiation was not strong enough to affect the temperatures of the seedbeds. The temperatures of all seedbeds were the same, varying from 18° by night to 22° C. by day.

The germination tests were replicated with various species and seed lots. In some tests stratified seed was used. Stratification was done by soaking the seed at 5° C. for 10 days.

Because the responses in germination varied greatly, the data were divided into several parts. The data for *B. pubescens* are represented in Table I. When data for the two treatments of 5 and 24 hr./day were analyzed alone, the effects of light and stratification were significant at $P < 0.05$. The following conclusions may be drawn from the results.

(*a*) The difference between light treatment means indicated that *B. pubescens* seed may germinate better under long photoperiods than under

TABLE I

INFLUENCE OF STRATIFICATION AND PHOTOPERIODS ON GERMINATION OF *Betula pubescens* SEED (IN POTS UNDER INDIRECT WEAK SUNLIGHT AND FLUORESCENT TUBES AT 19° TO 20° C. EXPT. 1)

	Germination in 30 days, %			
	No light	Indirect sun 8 hr./day	Indirect sun + fluorescent 5 hr./day	Indirect sun + fluorescent 24 hr./day
Not stratified	0	0.3	2	31
Stratified for 10 days	0.6	6	24	57

Source of variance	Degrees of freedom	Mean square	*F* ratio
Stratification	1	1227	25†
Light treatments	3	1197	24†
Repl. (= seed lots)	2	248	4.9*
Intera. strat. × light	3	94	1.9
Intera. light × repl.	6	89	1.8
Residual	8	50	
Total	23		

*$P < 0.05$.
†$P < 0.01$.

short photoperiods or in darkness. This experiment does not provide definite information on the nature of the responses, but large significant differences between 5 and 24 hr. treatments suggest true photoperiodic responses.

(*b*) The quality or quantity of the illumination may also influence germination because the addition of fluorescent tubes, even with a reduction in the duration of light, increased germination significantly.

(*c*) A significant ($P < 0.05$) effect of stratification was also demonstrated. The stratification for 10 days induced only a partial stimulation in germination which was still further enhanced by light; in other similar tests with stratification for four weeks, this seed germinated well even in continuous darkness.

(*d*) The small interaction light treatments × replications (= seed ages or sources) suggested that the response of birch germination to light does not vary greatly among different seed lots.

(*e*) Germination tests in dim rooms in winter may give grossly erroneous ideas of the germination of certain seed.

The results with one seed lot of *B. verrucosa* were similar to those with *B. pubescens*. This and the following experiments suggest that the above conclusions are applicable also to *B. verrucosa*.

The germination of birch began early and reached its peak early under the same conditions where final percentages were high. The continuous light not only gave the highest cumulative germination but also the speediest germination.

When the test period was extended over the usual germination period, no new germination took place. This indicates that the inhibition of germination under short photoperiods could not be overcome by added cumulative effect of light as such. On the other hand, when some seed lots were transferred from short photoperiods to long ones after the usual period, new germination immediately took place; nearly the same germination percentages were finally reached as with the seeds kept under long photoperiods all the time. This strongly suggests that a true photoperiodic phenomenon occurred.

Table II shows the responses of very old seed of *P. sylvestris*. This germinated poorly and slowly. The effect of stratification, if any, was suppressing rather than stimulating. Other (unpublished) tests showed that stratification has usually small or no effects on the germination capacity of this species. The confidence limits were rather low, and the difference between the means of the 24 and 5 hr./day treatments failed to be significant at $P < 0.05$. Both these means were different from those of the other two treatments at $P < 0.05$.

The remaining part of the data dealt with one seed lot of each of *Pinus sylvestris* (fresh seed), *Picea abies*, and *Alnus incana* (L.) Moench. Because these data were heterogeneous, the possible small responses could not be statistically established (at $P < 0.05$) in the same design as with the other seeds. However, an interesting feature was the order of the germination. With all these species, the highest germination was in the treatment 24 hr./day, and the next highest in the treatment 5 hr./day (with fluorescent

TABLE II

INFLUENCE OF STRATIFICATION AND PHOTOPERIODS ON GERMINATION OF OLD SEED OF *Pinus sylvestris* (IN POTS UNDER INDIRECT WEAK SUNLIGHT AND FLUORESCENT TUBES AT 18° TO 20° C. EXPT. 1)

	Germination in 35 days, %			
	No light	Indirect sun 8 hr./day	Indirect sun + fluorescent 5 hr./day	Indirect sun + fluorescent 24 hr./day
Not stratified	11	13	23	33
Stratified	9	5	14	27

Source of variance	Degrees of freedom	Mean square	*F* ratio
Light treatments	3	109	27*
Stratification	1	42	15*
Residual	3	4	
Total	7		

*$P < 0.05$.

lights), though the differences were small; the dim room light of natural day length and darkness gave the lowest germination. The consistency in these results suggests that real differences existed.

The results of Expt. 1 with five tree species may be summarized as follows: If tested in cool rooms in winter, seeds may germinate better under continuous fluorescent lighting than under short light periods or in darkness. The responses of spruce, pine, and alder may exist but are small. Birch (*B. pubescens* and *B. verrucosa*) will respond very markedly. If stratified, the need of light for germination of birch seed is reduced.

Factors Interacting with Photoperiods

Experiment 2

Seed of *B. verrucosa* was germinated on sterile sand in pots in growth chambers. By means of fluorescent tubes and screens, light conditions were arranged as shown in Table III.

The pots were kept standing in large, shallow containers, through which water of the desired temperatures was kept running. Air temperatures were automatically controlled and varied from 10° C. by night to 20° C. by day. Measurements with small thermocouples indicated that temperature differences in the thin surface layers of seedbeds were so small (1° C.) that they hardly could affect the germination.

Table III shows the results. These differed from those of Expt. 1 by having no germination at all under the shortest photoperiods. This is assumed to be due to the lower temperature in Expt. 2. Again both the illumination intensity and photoperiods largely influenced germination. The effect of

TABLE III

INFLUENCE OF ILLUMINATION INTENSITY AND PHOTOPERIODS ON GERMINATION OF *Betula verrucosa* SEED (IN POTS IN GROWTH CHAMBERS UNDER FLUORESCENT TUBES AT 10° TO 20° C. EXPT. 2)

	Germination in 30 days, %				
Intensity,* ft.-c.	8 hr./day	12 hr./day	18 hr./day	24 hr./day	Mean
20	0	1	20	18	10
300	0	4	30	52	24
Mean	0	2.5	25	35	17

Source of variance	Degrees of freedom	Mean square	*F* ratio
Photoperiods	3	1192	119†
Light intensity	1	239	24†
Interaction	3	92	9†
Residual	16	10	
Total	23		

**Germination in darkness averaged 1% and was not included in analysis.*
†$P < 0.01$.

photoperiods was, however, much larger. More than a 10-fold increase in light intensity (from 20 to 300 ft-c.) increased germination less than a small increase in photoperiods (from 12 to 18 hr./day).

Experiment 3

Seed of *B. verrucosa* was germinated on blotting paper in Petri dishes that were floating in water baths. In one set the water bath was cooled by running tap water. By utilizing direct sunlight from the southeast window, the following light conditions were arranged:

(I*a*) 24 hr. Direct sunlight for six hours from 8 a.m. to 2 p.m. with maximum intensity of 3000 to 4000 ft-c.; indirect sunlight for five hours from 2 p.m. to 7 p.m. with maximum intensity of 50 ft-c.; fluorescent light of 12 ft-c. all the time.

(I*b*) As (I*a*) but with light intensities being 20% of those in (I*a*).

(I*c*) As (I*a*) but with light intensities of about 0.1%.

(II*a*) 9 hr. As (I) including all the direct sunlight but only three hours from the indirect light.

(II*b*) As (II*a*) but with light intensities of 20%.

(II*c*) As (II*a*) but with light intensities of about 0.1%.

Because of significant large interactions the analysis was divided into two parts (Table IV). At the lower temperature range, long photoperiods gave significantly ($P < 0.05$) higher germination than did the short photoperiods but the difference was small. The germination was fair even under short photoperiods. This difference from the results in Expt. 2 was thought to be

TABLE IV

INFLUENCE OF TEMPERATURE, ILLUMINATION INTENSITY, AND PHOTOPERIODS ON GERMINATION OF *Betula verrucosa* SEED (IN PETRI DISHES IN WATER BATHS UNDER DIRECT SUNLIGHT AND FLUORESCENT TUBES. EXPT. 3)

Temperature range, ° C.*	Maximum intensity, ft-c.	Germination in 18 days, %		
		9 hr./day	24 hr./day	Diff. (24−9)
10 to 25	Approx. 2	33	50	17
	800	35	56	21
	4000	53	58	5
20 to 35	Approx. 2	49	43	−6
	800	52	52	0
	4000	10	18	8

Significant effects: (1) (At 10° to 25° C.): the effect of photoperiod at $P < 0.05$. (2) (At 20° to 35° C.): the effect of illumination intensity at $P < 0.01$.
**The temperature of the water surrounding the dish.*

due to the higher temperature in Expt. 3. The same appeared true in several other experiments performed with various methods at temperatures reaching well above 20° C. (e.g. Expts. 5 to 8).

At the higher temperature range (Table IV) the responses to photoperiods were not significant at $P < 0.05$. The effect of illumination intensity (Table IV) was obviously due to either the maximum radiation or the temperatures that were supraoptimal to germination. The cooling may not have been effective enough to prevent the rise of temperature in the fully exposed dishes more than in the others. No temperature measurements were actually made in the dishes.

Experiment 4

Seed of *B. verrucosa* was germinated on sterile glass wool in Petri dishes in an incubator at 35° C. Half of 20 seed lots were covered with black papers, and half were exposed to fluorescent illumination of 150 ft-c. The germination of covered seed was lower by only $0.5 \pm .03\%$. This difference is negligible and the result thus suggested that there is no effect of light at this temperature.

Experiments 5 and 6

These were performed in the same way as Expt. 1 and 2 but at higher temperatures, between 18° and 25° C. The effects of photoperiods were significant (at $P < 0.05$) but small. The results confirmed the earlier findings that the strong photoperiodic responses of birch seed take place only at low temperatures. Since no new conclusions were made, the details are not presented here.

Summary of Experiments 3 to 6

High germination of birch takes place at temperatures reaching 25° to 35° C. without long photoperiods and even in darkness. At moderate temperatures of 18° to 25° C. fair germination takes place even under short photoperiods.

At still lower temperatures long photoperiods are needed for high germination of unstratified birch seed. The effect of light intensity is less decisive but under certain conditions it is correlated with germination. Strongest illumination may decrease germination.

Germination Tests in Running Water

Experiment 7

Seed of *B. pubescens* was germinated in running water at 18° to 24° C. and exposed to daily photoperiods of eight hours without and with an additional 10 min. light period at night. The seeds were kept submerged in thin wire netting envelopes.

Table V shows the results. The midnight flash significantly ($P < 0.05$) increased germination while a 10-fold increase in light intensity failed to stimulate germination significantly. This is again a proof that a type of response exists that cannot be explained by the increased amount of light. On the contrary, the response was similar to the photoperiodic induction of flowering of long-day plants which is known to be initiated by a midnight flash.

TABLE V

INFLUENCE OF DIVIDING THE LONG DARK DAILY PERIOD INTO TWO SHORT ONES BY AN ADDITIONAL PHOTOPERIOD OF SHORT DURATION, ON GERMINATION OF *Betula pubescens* SEED (IN AERATED RUNNING WATER UNDER FLUORESCENT TUBES AT 18° TO 24° C. EXPT. 7)

	Germination in 21 days, %		
Intensity,* ft-c.	(A) 8 hr./day + 10 min./day	(B) 8 hr./day	Diff. (A − B)
300	31.2	27.8	3.4
30	29.6	22.4	7.2

Source of variance	Degrees of freedom	Mean squares	*F* ratio
Photoperiods	1	146	4.6†
Light intensity	1	58	1.8
Residual	17	32	
Total	19		

**Germination in darkness averaged 4.4% and was not included in analysis.*
†$P < 0.05$.

Experiment 8

Seed of *B. verrucosa*, contained in wire baskets, was floated on the surface of running cool water. The light conditions were as shown in Table VI. Because of the effective cooling even the strong light did not affect the temperatures significantly.

Despite the temperature being as low as 12° to 13° C. (occasionally 15° C.), there was fair germination already in seven days under proper light conditions. To illustrate the whole rate of germination, cumulative germination percentages from six contrasting conditions are shown in Fig. 1.

TABLE VI

INFLUENCE OF ILLUMINATION INTENSITY AND PHOTOPERIODS ON GERMINATION OF *Betula verrucosa* SEED (FLOATING ON THE SURFACE OF RUNNING WATER UNDER FLUORESCENT TUBES AT 12° TO 15° C. EXPT. 8)

Illumination intensity,* ft-c.	Germination in 7 days, %			
	12 hr./day	6 hr. twice a day	24 hr./day	Mean
10	10	20	24	18
40	13	29	34	26
200	20	32	36	29
1000	8	25	16	16
Mean	13	27	27	19

Source of variance	Degrees of freedom	Mean square	*F* ratio
Photoperiods	2	375	63†
Light intensity	3	109	18†
Interaction	6	16	2.7
Residual	12	6	
Total	23		

**Germination in darkness was about 0% and was not included in analysis.*
†P <0 01.

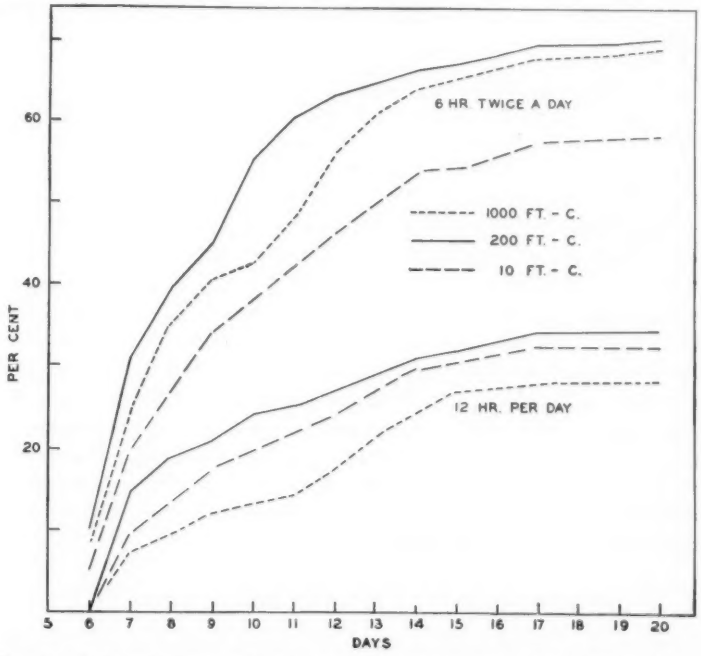

FIG. 1. Germination rate of *Betula verrucosa* seed as affected by photoperiods and illumination intensity. Seed floating on the surface of running water under fluorescent tubes at 12° to 15° C. Expt. 8.

The germination was slow under the illumination intensity of 1000 ft-c. (difference significant at $P < 0.05$). This is in accord with the results of Expt. 3 so far that in both instances a supraoptimal intensity appeared to be reached. Fig. 1 suggests that the high illumination intensity was tolerated better under optimum than under poor photoperiodic conditions. The germination was slow also under the light intensity of 10 ft-c. ($P < 0.05$) (Table VI, Fig. 1). The responses to photoperiods of 24 hr./day and six hours twice a day did not differ significantly ($P < 0.05$) from each other. Both results were significantly ($P < 0.01$) higher than with photoperiod of 12 hr./day. This is an additional proof that the photoperiodic responses in germination were phenomena similar to the photoperiodic induction of flowering of long-day plants and to the stimulation of vegetative growth in short-day plants. In these phenomena the more effective factor actually is the length of continuous dark periods. The results indicated that the further shortening of the dark periods from six hours to zero (compare the treatments six hours twice a day and 24 hr./day) does not change germination. The dark periods of 12 hr. in the treatment 12 hr./day allowed quite low germination only.

Effect of an Antibiotic

Since the disease resistance of birch seed appears to be low under poor light conditions (16), the possibility remains that the photoperiodic response is caused by a disease carried in the seeds. To explore this, the seeds were put to germinate in an antibiotic solution. The antibiotic Rimocidin was chosen since it was found harmless to birch seed and very effective against fungal diseases (17). The experiment was arranged in the same way as Expt. 8 except that instead of in wire baskets the seed was floated on Rimocidin solution (50 p.p.m.) or on water in small aluminum vessels. The vessels were floated on running cooling water at 14° to 17° C.

Rimocidin had no significant ($P < 0.05$) effect on germination while the response to photoperiods was significant ($P < 0.01$). Consequently, the hypothesis on the disease on seeds was not substantiated.

The results again demonstrated that dividing the otherwise long (18 hr.) daily dark period into two short dark periods (of nine hours) with an intervening short (two hours) light period may greatly stimulate germination even though the light period is short (four hours). It can be concluded that the critical length of the effective dark periods was about nine hours or more. In natural light conditions this means days 15 hr. long.

Treatments with Chemicals and Ultrasonics

For nursery practice a treatment is needed that would induce germination of birch seed even when it is put in darkness under a cover of seedbed soil. Stratification serves this purpose but is inconvenient to use.

Since seed often will be treated with various biologically active chemicals, some miscellaneous chemicals were mixed in the germination media. Each of the following chemicals was tried at varying concentrations: ammonium sulphate, ascorbic acid, benzoic acid, choline chloride, captan, dextrose, ethanol, ferbam, gallic acid, glycine, heptachlor, lauryl sulphate, lindane, maleic acid, malachite green, methanol, nabam, naphthalene acetic acid, picric acid, nicotinic acid, potassium nitrate, potassium phosphate, pyridoxine hydrochloride, Rimocidin, salicylic acid, sodium hypochlorite, succinic acid, sulphapyridine, sulphuric acid, thiamine, thiram, and zineb. None of these induced seed of *B. verrucosa* to germinate in darkness at 13° C.

The same seed was also treated with ultrasonic vibrations because these are known to affect biological processes. The treatments with cycles of 10,000 using 0.9 ampere current lasted from 1 to 120 min. No significant stimulation of germination was found.

Discussion

The writer's earlier observations (16) that day length influences germination of seed was confirmed by the present study. Long daily photoperiods stimulate germination of *B. verrucosa* and *B. pubescens* at temperatures below 20° C.; other species also appear to be stimulated but to a lesser degree.

In this study evidence similar to that reported by Black and Wareing (3) for *B. pubescens* showed that the responses in germination of two birch species are true photoperiodic phenomena. It was found that the inhibition of germination is primarily correlated to the length of the dark periods but not very closely to the amount of light.

Many observations (especially Expt. 8) indicated that germination of birch was also correlated to the illumination intensity but this effect was less pronounced than the photoperiodic responses. Germination of *B. verrucosa* was slowed somewhat when the illumination intensity was either reduced or raised from a steady intensity of 200 ft-c. in Expt. 8 or from a peak of 4000 ft-c. (from sun) in Expt. 2. It appears that the natural sunlight, which in summer reaches the intensity of 10,000 ft-c., is supraoptimal for germination of birch seed if it is not shaded or covered. Full exposure also hinders successful rooting of germinating birch seed on natural media (17).

Good germination of Finnish *B. verrucosa* seed takes place at low temperatures if the day length is anywhere between 24 hr. and 15 hr. or perhaps somewhat shorter (Expts. 2 and 10). That is the day length from the end of April to the end of August at the place of origin of the seed. The photoperiodic responses may have ecological importance to the species by keeping the freshly fallen seed dormant until spring. Although temperature often would allow germination and growth between September and December, this time of year would be very unfavorable to the establishment of the seedlings. Similarly, the response to the light intensities may prevent some of the seed from germinating under conditions that are too exposed or so shaded that diseases are encouraged (16).

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THE PHYSIOLOGY OF HOST–PARASITE RELATIONS

I. THE ACCUMULATION OF RADIOACTIVE SUBSTANCES AT INFECTIONS OF FACULTATIVE AND OBLIGATE PARASITES INCLUDING TOBACCO MOSAIC VIRUS[1]

By Michael Shaw and D. J. Samborski[2]

Abstract

Excised, parasitized leaves were placed with their bases in radioactive solutions or exposed to radioactive carbon dioxide and the uptake and distribution of the tracers followed by autoradiography. Carbon dioxide and 20 other carbon compounds, including sugars, amino and organic acids, phenols, and indoleacetic acid, and/or their metabolic products, as well as phosphate and calcium, accumulated strongly at uredial and conidial colonies of the obligate parasites *Puccinia* and *Erysiphe*, respectively. Accumulation of glucose occurred in the host tissue at the sites of mildew colonies on leaves from which the ectoparasitic fungal mycelium had been removed, paralleled the respiration rate, increased progressively with the development of the parasites, and was most marked at type 4 infections. It was inhibited by sulphur dust (with mildew), sodium azide, 2,4-dinitrophenol and, reversibly, by anaerobiosis and was, therefore, dependent on aerobic respiration. On the other hand the tracers did not accumulate within senescent rust and mildew colonies or within the necrotic lesions produced by bacterial and fungal parasites which kill the tissues of their hosts. Radioactive glucose did not, but calcium and phosphate did, accumulate within young local lesions of tobacco mosaic virus on *Nicotiana*. The tracers did not enter dead areas on mechanically-wounded leaves and only accumulated at fresh wounds under conditions conducive to a high rate of water loss from the damaged surface. In conjunction with more conventional methods, tracer techniques provide a powerful method of attack on the problems of host–parasite relationships.

Introduction

Infection with both obligate and facultative plant parasites is followed by marked increases in the rate of respiration of the host tissues. On the other hand, photosynthesis, while it may or may not be stimulated at first, always decreases within a few days after infection (for references see (3)). With rusts and powdery mildews, increased respiration and decreased photosynthesis are accompanied by the accumulation of various substances, such as starch, in the host cells at the infection (1), by marked local increases in dry weight (11, 17), and, with the pycnial, aecial, and telial stages of many rusts, by localized hypertrophy. Localized hypertrophy has been reported also for the uredial stages of certain rusts (18). As rust and mildew infections progress, visible chlorosis may give place to the resynthesis of chlorophyll with the formation of 'green islands' (1). Colonies of both rust and mildew may be maintained in a condition of active sporulation for some time by feeding sugars to detached, infected leaves in total darkness. Under these conditions the host tissue at infections may flourish long after the remainder of the leaf has become moribund. The authors have noted 'green island'

[1]*Manuscript received December 30, 1955.*
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formation in the absence of sporulation on detached, infected wheat leaves cultured on water, and Yarwood and Cohen (18) reported hypertrophy at uredial infections of *Uromyces* on bean under these conditions, even though sporulation did not occur.

These phenomena point to the mobilization of substrates from surrounding healthy tissue at rust and mildew infections, possibly under the influence of a diffusible substance or substances produced by one or both members of the host–parasite complex. Allen (2, 3) suggested that such a mobilization originated in metabolic changes rather than in a simple 'permeability' increase of the type described by Thatcher (14). Changes in permeability may be considered to be the result of alterations in metabolism. In a preliminary account (12) of a portion of the work which is described in this paper it was suggested that the transport of substrates to infections of rust and mildew was an 'active' process.

Tracer techniques have not been employed to any extent in the investigation of host–parasite relations. However, Gottlieb and Garner (7) showed that radioactive phosphorus, supplied as phosphate, accumulated at infections of *P. graminis tritici* on the first seedling leaf of wheat. In their experiments the isotope was fed to the plants, in culture solutions, for several days. The specific activity of the uredospores was less than that of either healthy or infected leaf tissues, suggesting, though not proving, that the phosphorus accumulated in the host tissue as well as in the fungus. Allen (2) reported that the phosphorus accumulating at infections of mildew was not in the form of soluble organic phosphates. Cutter (6) proposed that phosphorylated compounds originating in the host tissue are utilized by obligate parasites. Atkinson and Shaw (5), using cytochemical methods, found high concentrations of acid phosphatase in association with the haustoria of mildew on barley. They postulated that the phosphatases might play an integral role in the utilization of organic phosphates by the parasite.

Yarwood and Jacobson (19, 20) have investigated the accumulation of radioactive hydrogen sulphide, sucrose, and phosphate in leaves infected with both facultative and obligate parasites, including tobacco mosaic virus. In their experiments detached leaves were placed in an atmosphere containing radioactive hydrogen sulphide and attached or detached leaves or shoots were immersed in solutions of radioactive sucrose or phosphate. Exposures to the isotopes lasted for from 8 to 91 hr.

As a first step in the investigation of the physiology of host–parasite relations with particular reference to *Puccinia graminis tritici* Erikss. and Henn. and *Erysiphe graminis hordei* El. Marchal we have investigated some of the factors governing the distribution of various radioactive carbon compounds, including carbon dioxide, as well as radioactive phosphate and calcium after uptake by leaves bearing infections of facultative and obligate parasites, including tobacco mosaic virus.

Methods

A. Exposure to Radioactivity and Autoradiography

The leaves or shoots, with their petioles or bases in water or buffer, were exposed to radioactive carbon dioxide (usually approximately 1 μc. per liter of air at a total carbon dioxide concentration of 0.03–0.05%) in both light and darkness, using the method described by Shaw and Maclachlan (13). A period of approximately one hour in darkness was allowed to elapse before introduction of the tracer in dark experiments in order to avoid any effects of preillumination on dark fixation. In the standard experiments the duration of exposure to the radioactive carbon dioxide was 20 min. In other experiments, with a modified apparatus, exposure periods of from one to five minutes were employed.

Three methods were used to administer tracers in solution. In Method I sections of wheat leaves 2–3 cm. long were shaken in buffered, radioactive solutions in Warburg flasks in darkness, oxygen consumption or carbon dioxide evolution being recorded. This method was adopted in examining the effects of anaerobiosis and respiratory inhibitors on uptake of radioactive glucose. At the end of the experimental period the leaf-pieces were removed from the Warburg flasks, washed thoroughly with water, and killed as rapidly as possible. In Method II the cut ends of excised leaves or shoots were placed in vials of the buffered, radioactive solutions; in Method III the solutions were injected directly into attached leaves or shoots with a hypodermic needle. The periods allowed for uptake and distribution of the tracer were varied from 20 min. up to 24 hr. depending on the information desired. The progress of uptake was followed by taking subsamples at intervals. Method II had the advantage of simplicity and gave results which were qualitatively identical with those obtained using attached leaves. All the results presented were obtained by Method II unless otherwise stated. The concentrations of the solutions used varied from 0.25 to 1% approximately and the activities were usually in the range from 5.0 to 0.1 μc. per ml.

After exposure to radioactivity the leaves were laid flat on pads of filter paper between 3/16 in. iron plates which had been preheated to 90°–95° C. Drying was completed at room temperature and heating was sometimes omitted. In preliminary tests no differences in distribution of radioactivity were noted between heated and unheated leaves. Wetting previously dried leaves with dilute hydrochloric acid to remove adsorbed carbon dioxide also had no effect on the distribution of radioactivity.

The standard radiographs were made on Kodak (No-Screen) X-ray film with exposure periods of from a few days up to three weeks. Microautoradiography (13) was also employed to determine the distribution of radioactivity in epidermal strips and in leaf sections. In exposures of up to a month in duration no fogging of X-ray or nuclear track emulsions was observed with either healthy or diseased leaves which had not been exposed to radioactivity.

For the figures showing the distribution of radioactivity contact prints were made directly from the original radiographs. Thus, *light areas indicate the*

sites of high radioactivity. Wherever it is stated in the text that the radioactive substance which was fed to the leaves became localized, it is implied that either the original substance or its products, or both, became localized.

B. Measurement of Radioactivity

Self-absorption is by no means complete in dried leaves and local variations in thickness cause corresponding variations in the intensity of radiographs, as was readily demonstrated by folding the lamina. A large number of determinations (11) showed that the dry weight of excised infections rose to a maximum of two to three times the dry weight of surrounding healthy tissue. Thus an increased intensity of fogging of the emulsions may be observed at infections even if there is no increase in the specific activity of the tissue. Actually, the 'accumulation' at infections described below much exceeded that due merely to increases in dry weight per unit area.

For determinations of specific activities the tissue was carefully dried in the oven at 95° C., and finely ground by hand in a small mortar. Counts were made, to 2% standard error, at infinite thickness in small dishes (diam.: 1 cm.) using a CFIS-2 gas flow counter (Atomic Energy of Canada Ltd.) and a proportional scaler (Model 183, Nuclear Instrument and Chemical Corp., Chicago).

C. Gas Exchange

Oxygen and carbon dioxide exchange were measured at 25 ± 0.02° C. using conventional Warburg techniques. The leaves were either cut into short lengths (2–3 cm.) with a razor or into disks (diam. 2.8 or 5 mm.) with a stainless steel cutter and floated on either phosphate (0.05 *M*) or phthalate (0.01 *M*) buffer usually at a pH of 5.5. Cylinder-nitrogen was passed over hot copper to remove oxygen before use. Respiratory quotients were measured by Warburg's direct method and a correction applied for retention of carbon dioxide in the buffer (15).

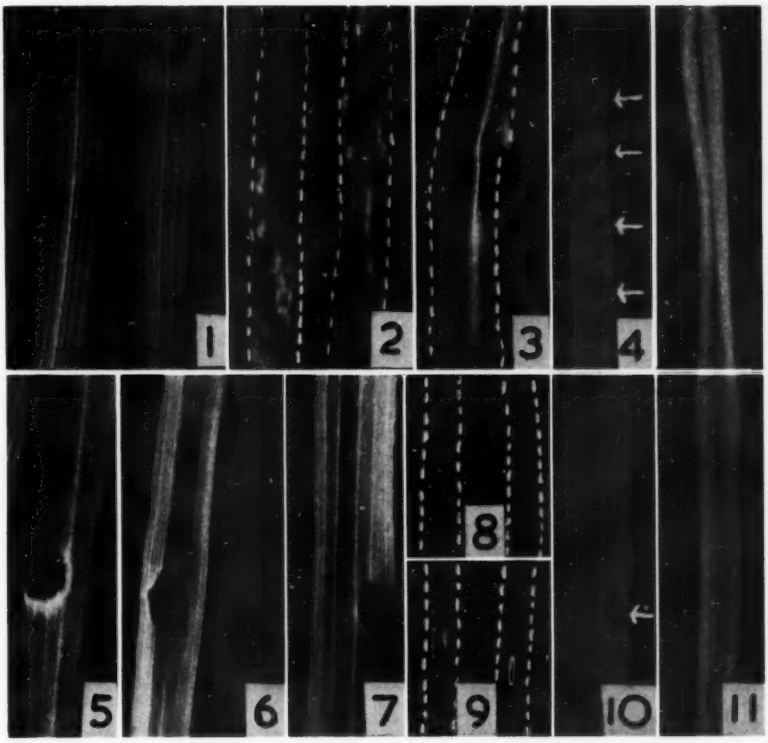
Plant Material

The list of host–parasite combinations employed is given in Table I. All infections were obtained by inoculation of greenhouse plants except for *Helminthosporium* sp. on *Bromus* sp. and *Phytophthora infestans* on potato, which were collected in the field. In all cases the experimental observations were carried out at several stages in the development of the disease.

Experimental Results

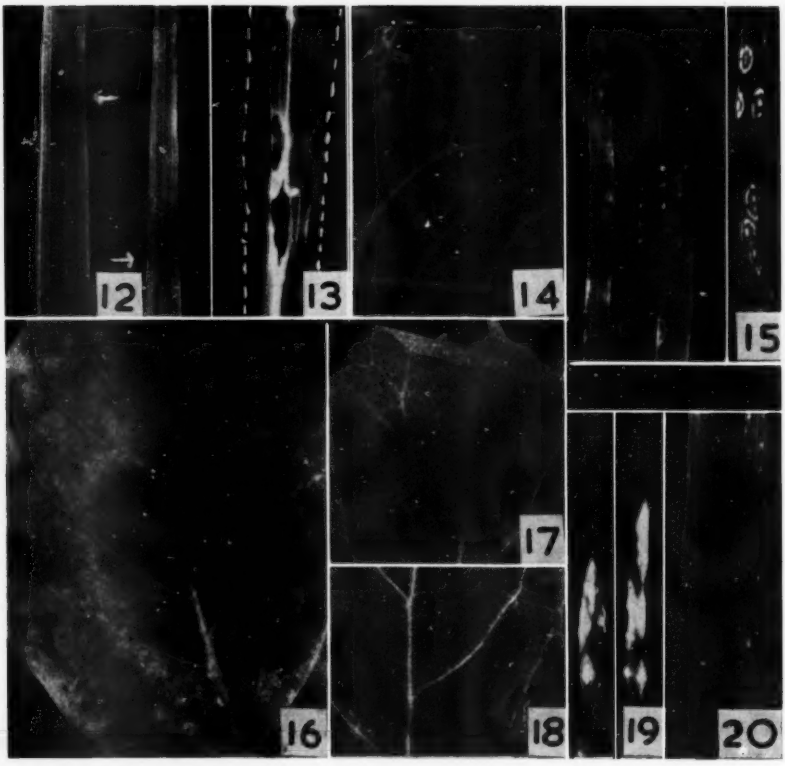
A. Wounding

The results of experiments on the effect of wounding on the distribution of radioactivity are summarized in Table II and Figs. 1 to 11. No radioactivity entered dead areas on the leaves and it was found that accumulation only occurred at freshly-inflicted wounds under conditions conducive to a high rate of water loss from the damaged surface. Thus a low water vapor pressure deficit or a vaseline seal prevented accumulation at fresh wounds.



FIGS. 1–11. Radiographs of leaves fed with 0.5% glucose-1-C^{14}. *Light areas indicate high radioactivity in these and all other radiographs.*

FIGS. 1 and 2. The effect of wounding healthy (FIG. 1) and mildewed (FIG. 2) leaves of barley by removal of epidermal strips. The leaves were kept at a low vapor pressure deficit. Wounding had no effect on the distribution of radioactivity. Accumulation occurred at the sites of some mildew colonies which had been removed by the stripping (FIG. 2). FIG. 3. The increase in radioactivity in the area from which the epidermis was stripped from a barley leaf kept at a high vapor pressure deficit. FIGS. 4, 10, and 11. The reduction of radioactivity at wounds made by pricking (FIG. 4), bruising (FIG. 10), and stripping (FIG. 11) wheat leaves which were kept at a low vapor pressure deficit. FIGS. 5, 6, and 7. The distribution of radioactivity at fresh (FIG. 5), two-day-old (FIG. 6), and three-day-old (FIG. 7) cork-borer 'wounds' on wheat leaves. There is some accumulation of activity near the cut surface in FIG. 5, but not in FIGS. 6 and 7. FIGS. 8 and 9. The accumulation of radioactivity at pricks (FIG. 9) and the inhibition of accumulation by vaseline (FIG. 8) on wheat leaves kept at a high vapor pressure deficit.



FIGS. 12–20. Radiographs of leaves fed with 0.5% glucose-1-C^{14} (xylose-1-C^{14} in FIG. 15).

FIG. 12. The reduction in radioactivity at lesions produced by *Pseudomonas coronafaciens* on oat leaves kept at a low vapor pressure deficit. FIG. 13. The accumulation of radioactivity around but not within old infections of *Helminthosporium* sp. on wheat. FIG. 14. Accumulation around infections of *Pseudomonas medicaginis* on bean. FIG. 15. Accumulation of radioactive xylose at eight-day-old rust infections on wheat leaves (var. Red Bobs) after 48, 90, and 165 min., from left to right respectively. FIGS. 16, 17, and 18. Lack of radioactivity in lesions produced by *Phytopthora* on potato (FIG. 16) and tomato (FIG. 17) or by *Botrytis* on bean (FIG. 18). There is some accumulation at the periphery of the infection zone in FIG. 16. FIGS. 19 and 20. Accumulation of radioactivity at normal 10-day-old rust infections on Little Club wheat (FIG. 19) and at 10-day-old infections on plants kept in continuous darkness for six days before feeding the tracer.

TABLE I

HOST–PARASITE COMBINATIONS EMPLOYED AS EXPERIMENTAL MATERIAL

Type	Organism and host
(1) Facultative	
(*a*) Bacteria	(i) *Pseudomonas medicaginis* f. sp. *phaseolicola* (Burkholder) Dowson; on *Phaseolus vulgaris* L.
	(ii) *Pseudomonas coronafaciens* (Elliot) Stapp; on *Avena sativa* L.
(*b*) Fungi	(i) *Botrytis* sp.; on *Vicia faba* L.
	(ii) *Helminthosporium* sp.; on *Bromus* sp. and on *Triticum dicoccum* Schübler
	(iii) *Phytophthora infestans* (Mont.) De Bary; on *Solanum tuberosum* L. and on *Lycopersicon esculentum* Mill.
(2) Obligate	
(*a*) Viruses	(i) Tobacco mosaic virus; on *Nicotiana glutinosa* L. and *N. tabacum* L.
	(ii) False stripe virus; on *Hordeum vulgare* L.
(*b*) Fungi	(i) *Puccinia helianthi* Schw.; on *Helianthus annus* L.
	(ii) *P. graminis tritici* Erikss. and Henn. (Races 15B and 56); on *Triticum dicoccum* Schübler (var. Khapli), *T. compactum* Host (var. Little Club) and *T. aestivum* L. emend. Fiori and Paoletti (var. Red Bobs)
	(iii) *Erysiphe graminis tritici* El. Marchal; on *T. compactum* Host (var. Little Club)
	(iv) *E. graminis hordei* El. Marchal. (Race 3); on *Hordeum vulgare* L. (vars. OAC 21, Peruvian and Macdonald College, Agronomy Department breeding selection 4709)

B. Facultative Parasites

The effects of facultative parasites on the uptake and distribution of glucose-1-C^{14} are shown in Figs. 12 to 14 and 16 to 18. In general there was markedly less radioactivity within the necrotic lesions than in surrounding healthy tissue. With *Pseudomonas medicaginis* on *Phaseolus* a moderate accumulation occurred at very young infections and around, but not within, older infections (Fig. 14). Some accumulation was also observed at the periphery of some, though not all, older lesions produced by *Helminthosporium* sp. on *Bromus* and on wheat (Fig. 13).

C. Viruses

Systemic infections of the false stripe virus on barley and of tobacco mosaic virus on *Nicotiana tabacum* L. did not affect the distribution of radioactive phosphate, glucose, or carbon dioxide in experiments lasting up to 20–24 hr. The distribution of these substances and of calcium was, however, affected at local lesions of the virus on tobacco. Typical results are shown in Figs. 21 to 29. Glucose-1-C^{14} did not enter lesions three to five days old, but accumulated at the peripheries and in the veins even where these crossed lesions (Fig. 22). In these experiments the distribution of radioactive glucose was similar to that of starch (Fig. 23). In short-term experiments (two to four minutes) with radioactive carbon dioxide a distinct depression of photosynthesis was evident at the lesions (Fig. 25). This was accentuated as the

TABLE II

THE EFFECT OF MECHANICALLY INFLICTED WOUNDS ON THE DISTRIBUTION OF RADIOACTIVE SUBSTANCES FED TO LEAVES

	Radioactive cpd.	Plant	Method of wounding	Activity at wound	Figure
Series I Low v.p.d.*	(1) $C^{14}O_2$ (20 min. in light)	Sunflower (Rusted and healthy)	Stripping	No effect	—
	(2) 0.5% glucose (4–6 hr.)	Wheat (Healthy)	Pricking	Reduced	4
			Stripping	Reduced or no effect	11
			Bruising	Reduced	10
	(3) 0.5% glucose	Barley (Healthy and mildewed)	Stripping	No effect	1 and 2
	(4) Very dilute phosphate (1–24 hr.)	Sunflower	Bruising	No effect	—
Series II High v.p.d.	(1) 0.5% glucose (16 hr.)	Barley (Mildewed and healthy)	Stripping	Increased	3
	(2) 0.5% glucose (20 hr.)	Wheat	Pricking, vaselined	No effect or slight reduction (32/38) leaves)	8
				Increased (6/38 leaves)	—
	(3) 0.5% glucose (20 hr.)	Wheat	Pricking, not vaselined	Increased (17/20 leaves)	9
				No effect (1/20)	
				Reduced (2/20 leaves)	
	(4) Very dilute calcium chloride (16 hr.)	Wheat	Bruising	Increased	—
Series III High v.p.d.	(1) 0.5% glucose (20 hr.)	Wheat	Cork borer		
			(*a*) Fresh wound	Increased	5
			(*b*) 2-day wound	Reduced or no effect	6
			(*c*) 3-day wound	Reduced or no effect	7
	(2) 0.5% glucose (20 hr.)	Sunflower	Cork borer		
			(*a*) Fresh wound	No effect	—
			(*b*) 2-day wound	No effect	—
			(*c*) 3-day wound	No effect	—

**v.p.d. = water vapor pressure deficit.*

lesions aged and became increasingly chlorotic. Results identical with those described were obtained with local lesions of the virus on *N. tabacum* L. (var. Chew Snuff.). On the other hand, radioactive calcium definitely accumulated in lesions on *N. glutinosa* (Fig. 28) and phosphate accumulated in lesions on *N. tabacum*. Phosphate was not fed to *N. glutinosa* and calcium was not fed to *N. tabacum*.

The results obtained with calcium and phosphate were apparently the opposite of those obtained with glucose and carbon dioxide. It can hardly be argued that the low activity in the lesions after feeding with glucose-1-C^{14} was due to oxidation and loss, as carbon dioxide, of the labelled carbon because the photosynthetic ability of the tissue within the lesions, though definitely reduced, was still high. Since these experiments were all performed at high light intensities of *ca.* 3000 ft-c., most of the radioactive carbon dioxide produced from glucose would have been refixed before escaping from the leaf.

Thus these results suggest either differential effects of the virus on permeability or on energy dependent mechanisms of transport and accumulation of carbon, calcium, and phosphate. However, they cannot be properly evaluated without much further work.

D. Rust and Powdery Mildew

A striking accumulation of radioactive substances was observed at vigorous colonies of these obligate parasites (Figs. 2, 15, 19, 47, 49, and 51). Little or no activity entered the severely injured or dead tissue at the center of senescent colonies. In addition to radioactive carbon dioxide, all the substances listed in Table III, or their metabolic products, were accumulated at infection sites, and in all cases activity became localized beneath uredia or mildew colonies after two to four hours. Protocatechuic acid was accumulated at infection sites in concentrations sufficient to cause visible damage after 18 hr.: healthy areas of the leaves showed no sign of injury. This observation is of interest from the standpoint of chemotherapy because it suggests that

TABLE III

RADIOACTIVE CARBON COMPOUNDS FED TO EXCISED, RUSTED, OR MILDEWED WHEAT OR BARLEY LEAVES

Class	Substance	Class	Substance
(1) Sugars	D-Glucose	(3) Acids	Benzoic acid ($C^{14}OOH$)
	D-Ribose		Propionic acid ($C^{14}OOH$)
	D-Xylose		Tyrosine (U.L.)
	D-Allose		Phenylalanine (U.L.)
(2) Sugar derivatives	D-Glucitol		β-Indoleacetic ($C^{14}OOH$)
	D-Gluconate (Cd salt)	(4) Phenols	p-OH-benzoic acid ($C^{14}OOH$)
	D-Mannitol (1-6-C^{14})		Protocatechuic acid ($C^{14}OOH$)
	l-Arabinate (Cd salt)		Vanillic acid ($C^{14}OOH$)
	D-Arabinate (Cd salt)	(5) Ions	Phosphate (P^{32})
	D-Lyxonolactone		Calcium (Ca^{45})
	D-Altronate (Cd salt)		
	D-Allonic-lactone		

All compounds with No. 1 carbon labelled unless otherwise indicated.
U.L. = *uniformly labelled.*

systemic antibiotics, poisons, or fungicides, present in the plant in concentrations too low to affect healthy tissue, might be accumulated at incipient infections in quantitites sufficient to limit further growth of the parasite. D-Lyxonolactone is not known to occur in nature. Particularly pronounced accumulation was observed with tyrosine and phenylalanine.

Work in our laboratory has shown that there are large increases in the concentration of free amino acids at rust infections. The transport of indoleacetic acid or its metabolic products to infection sites is of interest in the light of Pilet's (8, 9) observations on the increased auxin concentration in rusted leaf tissue. In our laboratory it has been found that there are increases in the indoleacetic acid content of the diseased parts of rusted and mildewed wheat and barley leaves. The origin of the excess indoleacetic acid is not yet known.

It is apparent from the foregoing that, at least initially, accumulation at infection sites bears little or no relation to the effect of the accumulated substance on the fungi. The influence on accumulation of light and darkness, age and type of infection, and respiratory inhibitors as well as the distribution of radioactivity in host and fungal tissues was investigated using both resistant and susceptible hosts.

Illumination, through its effect on transpiration, increased the rate of uptake of solutions by excised leaves. However, in six comprehensive experiments, illumination had no effect on the pattern of distribution of radioactivity or localization at infections. Results with glucose, ribose, xylose, and phosphate were the same. This indicates that the 'error' due to refixation of radioactive carbon dioxide produced from the sugars was negligible in these experiments. In less than two hours from the start of an experiment radioactivity was clearly localized beneath the uredia (Fig. 15).

The products of both 'light' and 'dark' fixation of carbon dioxide were also transported into and accumulated at infection sites of both *Puccinia* and *Erysiphe*, as shown for rust on sunflower in Figs. 30 and 31. Short-term exposures (one to five minutes) to carbon dioxide were employed to follow the movement of the tracer into infection zones. The results of one such experi-

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FIGS. 21–23. Photograph (orange filter), radiograph and photograph (after clearing and staining with iodine), respectively, of a single leaf of *Nicotiana glutinosa* bearing local lesions of tobacco mosaic virus. FIG. 21. Note slightly chlorotic four-day-old lesions. FIG. 22. Lack of radioactivity within lesions after the leaf was fed with radioactive glucose for six hours. Note moderate accumulation of radioactivity at the peripheries of the lesions. FIG. 23. Lack of starch within and its accumulation at the peripheries of the lesions.

FIGS. 24–26. Photograph (no filter), radiograph and photograph (after clearing and iodine staining), respectively, of a single leaf of *N. glutinosa* showing the depression of photosynthetic fixation of radioactive carbon dioxide (FIG. 25) and the distribution of starch (FIG. 26) at four-day-old lesions of tobacco mosaic virus. The leaf was exposed to radioactive carbon dioxide for approximately three minutes at a light intensity of *ca.* 3000 ft-c. and then killed immediately.

FIGS. 27–29. Photograph (no filter), radiograph and photograph (after clearing and iodine staining), respectively, of a single leaf of *N. glutinosa* showing the accumulation of radioactive calcium (FIG. 28) and the distribution of starch (FIG. 29) at four-day-old local lesions of tobacco mosaic virus. The leaf was fed with radioactive calcium for approximately six hours.

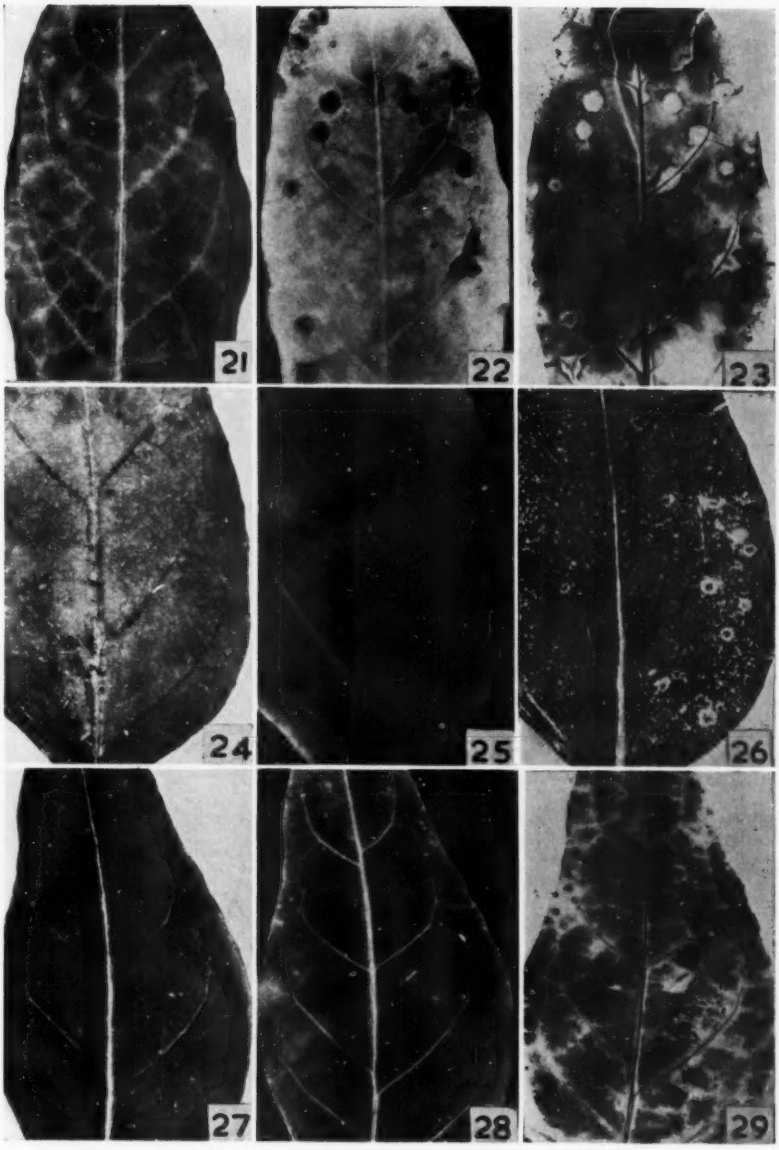
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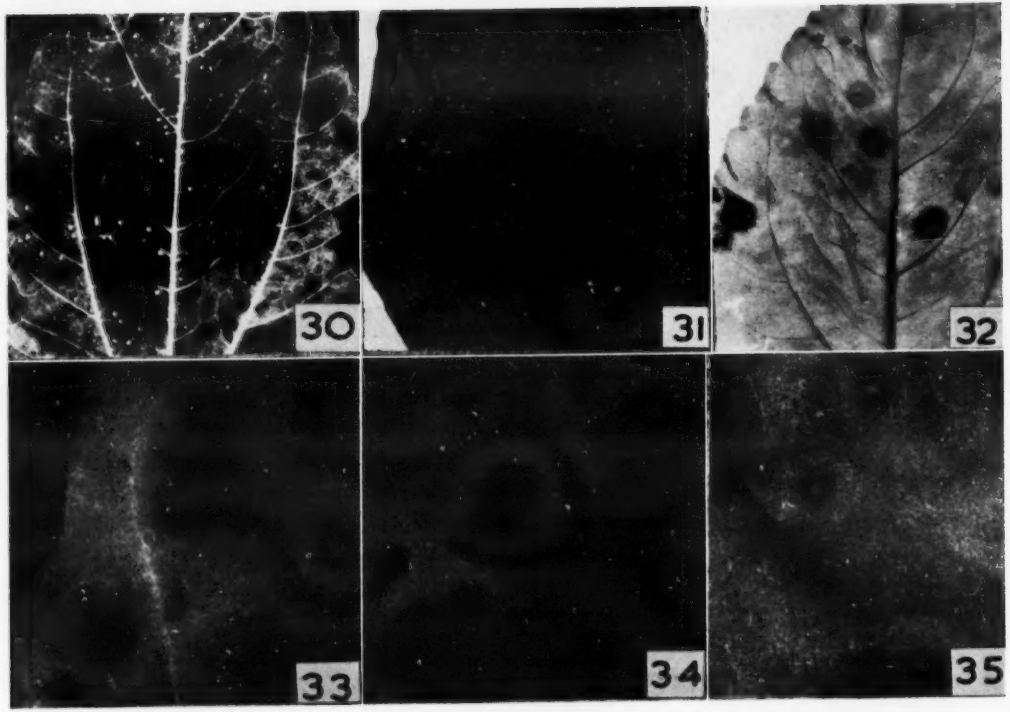
PLATE IV



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ment with rusted sunflower leaves are shown in Figs. 33 to 38. These illustrate the depression of photosynthetic fixation at the infection sites and the gradual increase in activity at the peripheries (Fig. 34) and centers (Fig. 35) of the pustules. Fig. 35 also illustrates the 'zonal' distribution of activity frequently observed at rust pustules. The Central Zone (I) corresponds to the position of developing uredospores and was highly active. Zone II showed less activity while Zone III was highly active. As may be seen from Fig. 32, Zone III was also high in starch content.

A depression of photosynthesis at infection sites was also observed, in exposures to radioactive carbon dioxide of one to three minutes' duration at light intensities of *ca.* 3000 ft-c., with mildew on barley and in some cases with non-erumpent wheat-rust infections five to six days old. In other short-term experiments there was a definite stimulation of fixation at non-erumpent infections four to five days old. Such seemingly contradictory results were also obtained with older infections and are illustrated for 14-day-old type 4 rust pustules in Figs. 36 and 37. Fig. 38 illustrates diagrammatically the depression of carbon dioxide fixation at primary and secondary uredia. All observations were compatible with the provisional conclusion that fixation is depressed at sporulation sites and stimulated in the surrounding zone. This peripheral stimulation is not evident if the rate of photosynthesis of more distant healthy areas is also high, as in the radiograph illustrated in Fig. 37, but is evident if the photosynthetic rate in these areas is low, as in Fig. 36. Possibly the stimulation in the zone immediately surrounding sporulation sites is due to a lowering of the resistance to inward diffusion of carbon dioxide, caused for example by injury to the epidermis. However, an actual biochemical stimulation cannot be ruled out.

The effect of age of infection on accumulation is illustrated for mildew on three varieties of barley in Figs. 39–42. There was no detectable localization of activity on inoculated leaves of the 'immune' variety 4709. With the other two varieties, accumulation was distinct at three days after inoculation. By the 12th day after inoculation, the leaves were dying off, and accumulation was much reduced. It is noteworthy that accumulation at the type 3 infections on variety Peruvian was considerably less marked than at the type 4 infections on variety OAC21 (see Fig. 42), although the size of the mildew

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FIGS. 30 and 31. Radiographs of sunflower leaves showing the accumulation or radioactivity in 10-day-old infections of *Puccinia helianthi* during 20-min. exposures to radioactive carbon dioxide in light (FIG. 30) and darkness (FIG. 31) respectively.

FIG. 32. Photograph illustrating the accumulation of starch at 10-day-old infections of *P. helianthi* on sunflower. The plant was kept in darkness for 16–20 hr. prior to decolorization and iodine staining.

FIGS. 33–35. Radiographs of sunflower leaves, bearing eight-day-old infections of *P. helianthi*, after a three-minute exposure to radioactive carbon dioxide at a light intensity of 500 ft-c. FIG. 33. Leaf killed immediately exposure ended. FIG. 34. Leaf killed after standing for 20 min. in normal air. Note increased activity at the peripheries of infection sites. FIG. 35. Leaf killed after four and one-half hours in normal air. Note the transport of radioactivity to the infection centers.

TABLE IV

THE QUANTITATIVE DETERMINATION OF THE ACCUMULATION OF GLUCOSE-1-C^{14} OR ITS PRODUCTS AT 10-DAY-OLD RUST INFECTIONS

Variety	Tissue	Counts/minute	Ratio R/H (Accumulation ratio)
Little Club	Healthy (H)	1206 ± 12	
	Rusted (R)	2691 ± 27	2.23
	R + H	3897 ± 23	
Khapli I	Healthy (H)	1637 ± 16	
	Rusted (R)	2200 ± 22	1.34
	R + H	3837 ± 23	

Healthy and rusted areas were cut from the same leaves after feeding with 0.5% glucose-1-C^{14} for 12 hr. The total activities taken up by the two varieties were almost identical.

colonies appeared to be similar in the two cases. Similar results were obtained with rust (Race 15B) on the wheat varieties Little Club (type 4 infection), Khapli I* (type 2–3⁻⁻), and Khapli (type 1).

The accumulation ratios for 10-day-old infections of Race 15B on Little Club and Khapli I wheats were 2.23 and 1.34 respectively (Table IV). In other experiments with glucose-1-C^{14}, carried out in Warburg flasks (Method I), rusted tissue accumulated 2.4 times as much activity as did healthy tissue cut from comparable but uninfected leaves (Table V). As judged from a comparison of the autoradiographs (not illustrated) of the leaves from which the data in Table IV were obtained with, for example, the radiograph illustrated in Fig. 19, very much higher accumulation ratios may occur and have in fact been reported for phosphate (20). The data in Table V also show that 'accumulation' of radioactivity in the tissue (C) was proportional to the respiration rate (A). However, per unit oxygen consumption (D/A) or per unit tissue activity (D/C), the No. 1 carbon of glucose was oxidized to carbon dioxide approximately 2.4 times as fast in rusted as in healthy tissue. One interpretation of this result is that the pathways of glucose-oxidation in rusted and healthy tissue are different. More detailed evidence bearing on this point will be presented in a later report.

Continuous darkness, which reduced rust development, reduced accumulation (Figs. 19 and 20). However, the radiographs of infected leaves taken from plants maintained in continuous darkness showed evidence of accumulation in the host tissue beyond the limits of the restricted mycelial development (Fig. 20).

The ectoparasitic mycelium of *Erysiphe* can be readily separated from its host by rubbing the entire surface of parasitized leaves with a cotton swab. From radiographs of leaves fed with radioactive glucose, both before (Fig. 54) and after (Fig. 53) removal of the mildew mycelium, it was concluded that the bulk of the activity which accumulated at infections appeared to be in the

**Khapli I designates an 'off type' Khapli maintained in the Department of Biology field plots.*

TABLE V

UPTAKE OF OXYGEN AND GLUCOSE-1-C^{14} AND EVOLUTION OF $C^{14}O_2$ BY HEALTHY AND RUSTED WHEAT-LEAF-PIECES

O_2 uptake per 10 mgm. D.W. (A)			R.Q. (B)		Activity in tissue (c.p.m.) (C)			Activity in CO_2 (c.p.m.)* (D)		D/C			D/A		
R	H	R/H	R	H	R	H	R/H	R	H	R	H	R/H	R	H	R/H
158	63	2.51	0.95	0.91	428	179	2.39	2618	454	6.12	2.54	2.41	16.57	7.21	2.30
161	65	2.48	0.95	0.95	418	167	2.44	2590	441	6.35	2.64	2.41	16.09	6.78	2.37

First leaves of Little Club wheat. Rust infections nine days old. Glucose-1-C^{14} conc. = 0.057%. Phosphate buffer pH 5.5. Duration of experiment 4.5 hr. Method I.

**Carbon dioxide trapped in the center wells was precipitated and counted as $BaC^{14}O_3$. The figures for activity in CO_2 and in the tissue should not be compared directly, because of differences in the geometry of the system used in counting.*

host tissue. Since the above technique does not remove the haustoria from within the epidermal cells, further experiments were carried out in which strips of the lower epidermis of leaves bearing mildew colonies were removed both before and after the leaves were fed with radioactive glucose. In the 'strip-before' experiments accumulation of the tracer in the mesophyll in positions corresponding to infection sites was not usually evident. This did, however, occur in a few cases (Fig. 2). In the 'strip-after' experiments accumulation was observed both at the colonies on the isolated epidermal strips and in the corresponding positions on the stripped, epidermis-free mesophyll. From the relative intensities of the appropriate radiographs it was again concluded that the bulk of the accumulated activity was in the host tissue. It should be remarked that this does not necessarily imply that the specific activity of the host tissue at the infections was greater than that of the fungal mycelium, since the leaf tissue (mesophyll minus lower epidermis) has a greater dry weight per unit area than the epidermis plus mildew colony. Specific activities were not determined.

Microautoradiography of mildew conidia and mycelium isolated from leaves after the feeding of radioactive glucose showed that the mycelium and young (i.e. developing) conidia became radioactive, while, as might be expected, mature conidia did not. A few microautoradiographs were made of microtome sections of rust-infected wheat leaves which had been fed radioactive glucose. Difficulties were experienced with the diffusion of radioactivity during fixation of the sections and the results will not be presented here. They did suggest, however, that the bulk of the radioactivity at the infections was in the host tissue and provided a clear demonstration of the accumulation of activity in developing uredospores and teliospores. Further work on the histological distribution of activity at the infections is necessary.

TABLE VI

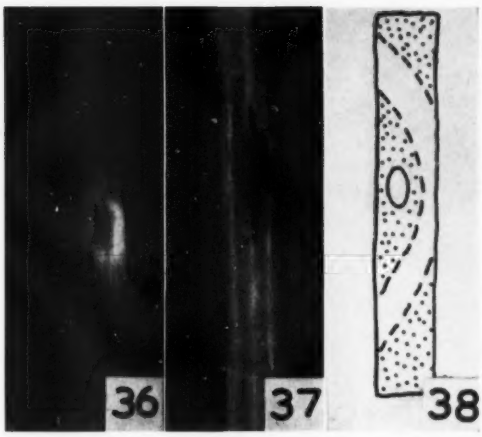
THE EFFECT OF ANAEROBIOSIS ON THE UPTAKE IN DARKNESS OF 0.5% GLUCOSE-1-C^{11} BY WHEAT-LEAF-PIECES BEARING EIGHT-DAY-OLD TYPE 4 RUST-INFECTIONS

Relative CO_2 output in 3 hr.		Relative activity taken up in 3 hr.		Accumulation at infections	
Air	N_2	Air	N_2	Air	N_2
100	18.2	100	25.2	High (Fig. 43)	Low (Fig. 44)

The data in this table were obtained by Method I.

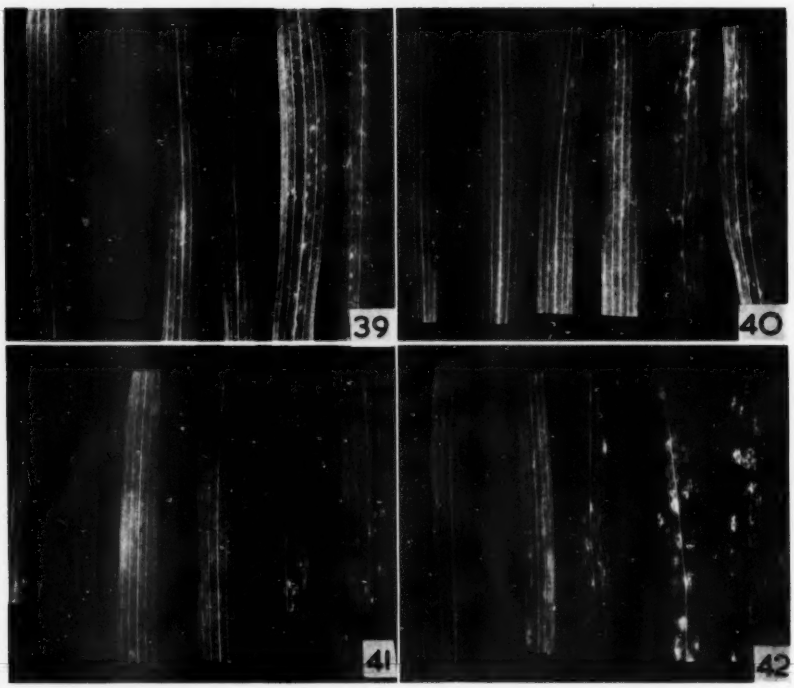
The data in Table VI and Figs. 43 and 44 illustrate the effect of anaerobiosis in inhibiting the uptake of radioactive glucose by wheat-leaf-pieces and its accumulation at rust pustules. Figs. 47 and 48 also illustrate this result. In a sample of leaves transferred to air, after four hours in a stream of nitrogen, accumulation occurred normally, indicating the reversibility of the inhibition. Anaerobiosis also inhibited the accumulation of calcium, though to a lesser extent than that of glucose.

PLATE V



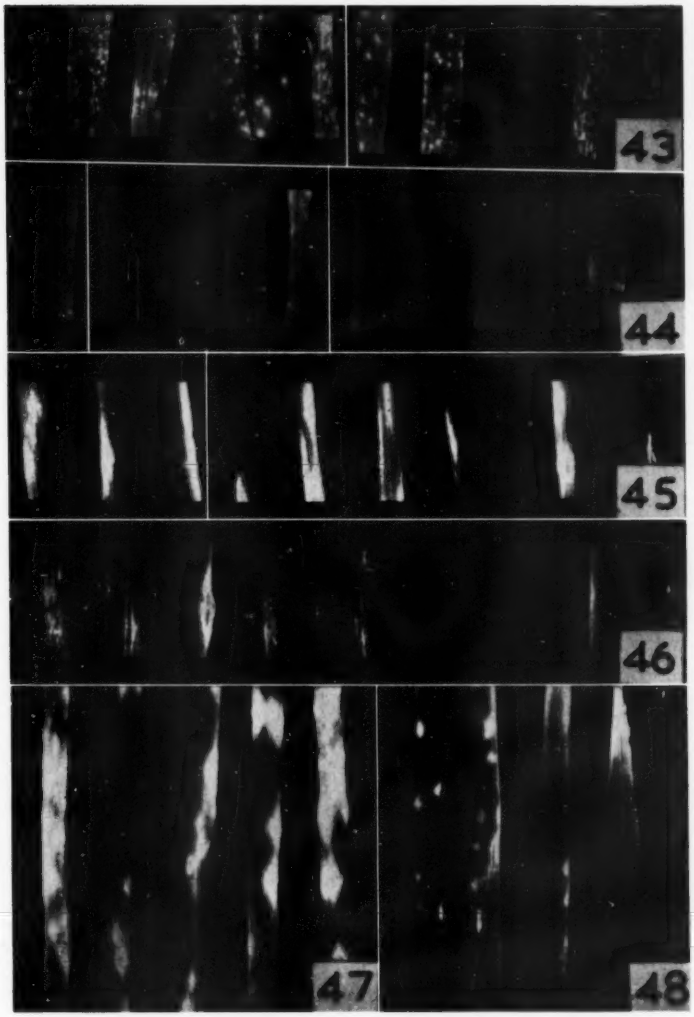
FIGS. 36 and 37. Radiographs of leaves of Little Club wheat, bearing 14-day-old rust infections, after four minutes in radioactive carbon dioxide at a light intensity of *ca.* 3000 ft-c. FIG. 36. Depression of fixation at infection center, 'stimulated' fixation at periphery of infection zone, and low fixation in healthy areas. FIG. 37. Depression of fixation at infection center and high rate of fixation in healthy areas.

FIG. 38. The distribution of radioactivity at a 14-day-old rust pustule on a leaf of Little Club wheat after 1.5 min. in radioactive carbon dioxide at a light intensity of 3000–4000 ft-c. The stippled areas showed high activity and the blank areas represent the sites of uredospore formation at which fixation was strongly depressed.



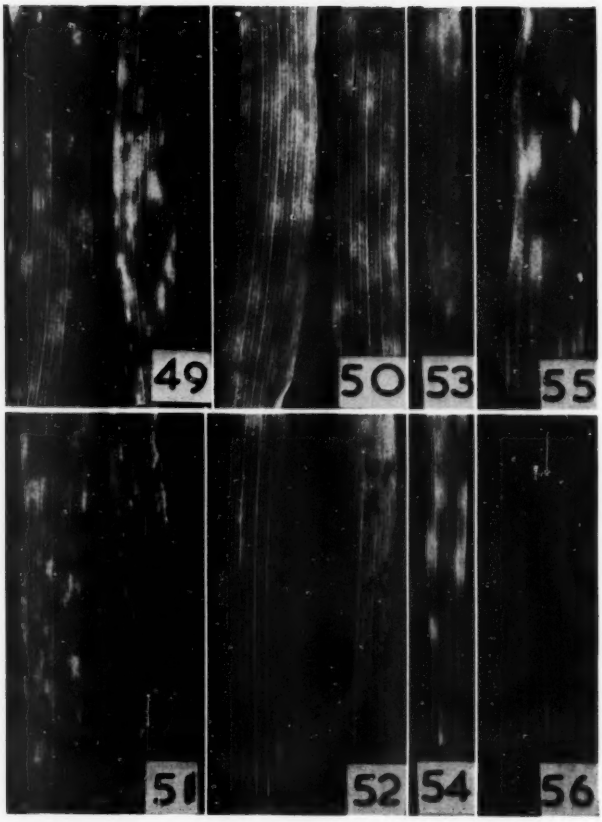
FIGS. 39–42. Radiographs of leaves of barley fed with 0.5% radioactive glucose for four to five hours at four days (FIG. 39), five days (FIG. 40), six days (FIG. 41), and eight days (FIG. 42) after inoculation with *Erysiphe graminis* (race 3). FIGS. 39–41 show two leaves of each of the varieties 4709, Peruvian, and OAC21, from left to right respectively. In FIG. 42 only one leaf of "variety" 4709 is shown. Note lack of accumulation on the 'immune' variety 4709. Accumulation at infections on the other varieties was distinct at four days after inoculation (FIG. 39) and increased until eight days after inoculation (FIG. 42), but was greater on variety OAC21 than on Peruvian.

PLATE VII



FIGS. 43–48. Radiographs of rust infected leaves of Little Club wheat fed with 0.5% glucose-1-C^{14}.

FIGS. 43 and 44. The accumulation of radioactivity at rust infections (FIG. 43) and the inhibition of accumulation by a nitrogen atmosphere (FIG. 44). This experiment was carried out with the leaf-pieces floating on glucose solution in Warburg flasks. FIGS. 47 and 48. The accumulation of radioactive glucose at rust infections (FIG. 47) and the inhibition of accumulation by a nitrogen atmosphere (FIG. 48). This experiment was carried out with the excised leaves maintained in a stream of air or nitrogen, respectively, in darkness. FIGS. 45 and 46. The accumulation of radioactive glucose at rust infections (FIG. 45) and the inhibition of accumulation by 10^{-5} *M* 2,4-dinitrophenol (FIG. 46).



FIGS. 49–52. The effect of sulphur dust on the accumulation of radioactive glucose at mildew infections on barley. FIGS. 49 and 51, control: no sulphur; FIG. 50, one day after sulphur treatment: note that localization of activity at infections is still clear; FIG. 52, three days after sulphur treatment, showing inhibition of accumulation.

FIG. 53. The accumulation of radioactive glucose at the sites of mildew colonies on barley. The fungal mycelium was rubbed off the leaf after the feeding of the tracer, but before the radiograph was made.

FIG. 54. The accumulation of radioactive glucose at the sites of mildew colonies on barley. The mildew mycelium was rubbed off the leaf before feeding of the tracer.

FIGS. 55 and 56. The inhibition by 10^{-5} *M* 2,4-dinitrophenol of the accumulation of radioactive glucose at infections of mildew on barley. FIG. 55, control leaves showing accumulation; FIG. 56, leaves infiltrated with 10^{-5} *M* dinitrophenol before feeding with tracer plus dinitrophenol.

The data in Table VII indicate the effect of 2,4-dinitrophenol and sodium azide on the rate of oxygen consumption and uptake of radioactive glucose. Uptake was inhibited by 60% and accumulation was strongly reduced by 10^{-5} *M* dinitrophenol, which stimulated oxygen consumption. Uptake and accumulation were even more strongly inhibited by 10^{-3} *M* dinitrophenol and 10^{-2} *M* azide. In two other experiments, the radioactivity of rusted leaf-pieces fed with glucose-1-C^{14} was reduced by 21% and 27% by 10^{-5} *M* dinitrophenol. Accumulation was also inhibited at infections on leaves pretreated by infiltration with 10^{-5} *M* (Figs. 55 and 56) and 10^{-3} *M* dinitrophenol. These and other data not recorded here indicate that the uptake of glucose by healthy and rusted wheat leaves is under enzymatic control. However, further work is necessary before the relative effects of dinitrophenol on the uptake, transport within the tissue, and oxidation of glucose can be evaluated.

TABLE VII

THE EFFECT OF DINITROPHENOL AND AZIDE ON THE ASSIMILATION OF GLUCOSE AND ON OXYGEN UPTAKE OF WHEAT-LEAF-PIECES BEARING 10-DAY-OLD TYPE 4 RUST-INFECTIONS

Treatment	Relative oxygen consumption in 1.5 hr.				Relative activity in tissue after 1.5 hr.	Accumulation at infections
	Initial rate in buffer*	+ Inhibitor	+ Glucose	+ I / + Gl		
0.5% Glucose-1-C^{14}	100	—	124.4	—	100.0	High (Fig. 45)
10^{-5} DNP	100	128.2	—	154.1	40.0	Much reduced (Fig. 46)
10^{-3} DNP	100	9.7	—	10.6	3.7	Not detectable
10^{-2} Azide	100	5.5	—	7.3	17.0	Not detectable

**Phosphate buffer, pH 5.5. The data in this table were obtained by Method I.*

Dusting with elemental sulphur is a well-known treatment for the control of mildew (16). In four experiments it was found, in agreement with (10), that pretreatment of mildewed leaves with sulphur dust did not reduce the oxygen consumption of excised leaf disks, but inhibited the accumulation of radioactive glucose at infection sites, as shown in Figs. 49 to 52. It is of interest that some accumulation of glucose occurred at the infections on the day following the application of sulphur (Fig. 50), by which time the fungus itself was presumed to be dead.

Discussion

The results of the experiments on the effect of mechanically-inflicted wounds indicate that the physical effects of an increased rate of water loss can cause the local accumulation of radioactive substances under suitable conditions. They also leave no doubt that such 'direct' effects of wounding play no more than a minor role in the accumulation of radioactive substances at infections of obligate parasites. It should be pointed out that marked accumulation would be expected to occur, through increased metabolic activity, wherever wounding initiates regeneration.

The main conclusion which can be drawn from the experiments carried out with facultative parasites is that, as expected, radioactive substances do not accumulate within infections in which the host tissue is already killed at the time the experiment is started. The moderate accumulation which sometimes occurs at young infections and at the periphery of older infections of facultative parasites may be due largely to a local increase in the rate of water loss, although accumulation through the enhanced metabolic activity of the host–parasite complex cannot be entirely ruled out.

The accumulation of radioactive substances (C^{14}, P^{32}, Ca^{45}) at infections of rust and mildew was not prevented by low vapor pressure deficits. Indeed, accumulation at such infections occurred on leaves enclosed in a saturated atmosphere and on leaf-pieces in Warburg flasks, even though in many of the experiments the leaves had been in contact with water long enough to attain full turgor before the tracers were added. In the experiments of Yarwood and Jacobsen (20) accumulation from solutions of radioactive phosphate occurred at infections of *Uromyces* on submerged bean leaves. In our experiments azide, dinitrophenol, sulphur, and anaerobiosis all strongly inhibited the accumulation of glucose-1-C^{14} at infections of *Puccinia* or *Erysiphe*. Unless it is argued that these agents act directly on cell permeability, there can thus be little doubt that the accumulation at infections is linked with their enhanced aerobic respiratory metabolism. An increased rate of transpiration at the infections may play a minor role under natural conditions. The general parallelisms between the degree of accumulation and stage of development of the parasite and the respiration rate are in accord with this conclusion.

There remains the question as to whether accumulation occurs primarily in the fungus or in the host tissue at infections of obligate fungal parasites. A simple view would be that the substances accumulate directly in the fungal mycelia and developing spores. However, the formation of starch in the host cells at infections of *Puccinia* and *Erysiphe* on wheat and barley, the hypertrophy which occurs at infections of some rusts (18), the increase in dry weight at infections, and the clear-cut demonstration that the enhancement of the respiration rate at infections of *Erysiphe* on wheat is due mainly to an increase in the respiration of the mesophyll of the wheat leaf (4) all speak against this conclusion. In addition it has now been shown that activity from glucose-1-C^{14} accumulated at the sites of mildew colonies from which the fungal mycelium had been removed (either before or after the feeding of the tracer) as well as in tracer experiments started 24 hr. after the mildewed leaves had been dusted with sulphur to kill the parasite. All these points, then, support the view that the various substances accumulate in the tissues of the host, whence they may become available to the parasite. Unfortunately, a separation of fungal and host tissues cannot be achieved in the case of the rust fungi. However, as mentioned above, preliminary results obtained by microautoradiography of sections of rusted leaves are not out of harmony with the conclusion reached

for mildew. The distribution of radioactivity on radiographs of rusted leaves in the 'time-course' experiments with radioactive carbon dioxide (Figs. 33–35) and sugar (Fig. 15) also supports this view. Finally, Yarwood and Jacobsen (20) have shown recently that the accumulation of radioactive phosphate at infections of *Uromyces* on bean, though somewhat reduced, is still clear at infections on leaves which were exposed to a temperature of 45° C. for 100 sec. to kill the fungus.

The results obtained by Yarwood and Jacobsen (20) are thus substantially in agreement with the foregoing. However, they found less P^{32} accumulation in diseased than in healthy areas of leaves collected in the field and bearing infections of a number of obligate parasites. The age of these infections was not stated, but unless there was either severely injured or dead tissue present, their results are contrary to those reported here for the uredial stages of *Puccinia* on wheat and sunflower, and for conidial colonies of *Erysiphe* on wheat and barley.

Yarwood and Jacobsen also observed accumulation of radioactive sulphur at injured and dead areas (killed with drops of sulphuric acid) on leaves exposed to radioactive hydrogen sulphide at a high relative humidity. Under these conditions, too, they found that sulphur accumulated at infections of several facultative parasites which are known to kill the tissues of their hosts. In view of these results indicating accumulation of sulphur in dead tissue and also of the known effects of hydrogen sulphide as a respiratory poison it does not seem safe to assume that the mechanism of accumulation of sulphur (supplied as hydrogen sulphide) is necessarily entirely comparable with that suggested here for the accumulation of radioactive carbon, phosphorus, and calcium. Moreover, Yarwood and Jacoben's radiographs show less radioactivity in the veins than in the interveinal areas, suggesting the possibility that sulphur was not translocated via the veins under their conditions. Possibly, the formation of sulphides of the heavy metals may play a role in the direct adsorption of hydrogen sulphide, particularly by injured or dead tissue.

The results obtained in the present work on the movement of radioactive carbon, phosphate, and calcium into local lesions of tobacco mosaic virus on tobacco, though difficult to interpret, are of particular interest, especially when compared with those obtained by Yarwood and Jacobsen using radioactive hydrogen sulphide. In our experiments young local lesions on leaves fed with glucose-1-C^{14} were strikingly less radioactive than the surrounding healthy tissue, although veins crossing the lesions were strongly radioactive. On the other hand there was a detectable, though moderate, accumulation of radioactive phosphate and calcium in young local lesions. In contrast to the above, Yarwood and Jacobsen observed a striking accumulation of sulphur from hydrogen sulphide at necrotic local lesions of tobacco mosaic virus on *N. glutinosa*. Again, it is unfortunate that they do not state whether the tissue was alive or dead at the time the experiment was started and whether or not exposure to hydrogen sulphide caused injury to the tissue at the infections.

Clearly, a full interpretation of both the results on tobacco mosaic virus reported here and those of Yarwood and Jacobsen must await further work. 'Time-course' studies of the type carried out with radioactive carbon dioxide (Figs. 33–35) would do much to determine whether the accumulation of sulphur at infections and wounds on leaves exposed to radioactive hydrogen sulphide is due to adsorption, perhaps by sulphide formation, or to a metabolically controlled assimilation and polar transport, or both. In the meantime, the results presented here for tobacco mosaic virus together with those for the fungal parasites serve to illustrate the value of the tracer technique as a powerful tool in the investigation of the relationships between localized disease lesions and the surrounding tissue.

The results obtained with *Puccinia* and *Erysiphe* are in full accord with the hypothesis that a diffusible substance (or substances), produced at the incipient infection, either by the fungus, or by the host cell or cells under attack, or both, stimulates the metabolic activity of the host tissue in the environs of the infections. The rapid transport of radioactive substances toward and their accumulation at infections is probably similar to the accumulation of radioactive substances in meristematic or other rapidly metabolizing regions. The fact that certain of the rust fungi cause a hypertrophy of the host tissue under natural conditions, and that the uredial stage of *Uromyces* causes a local hypertrophy of bean leaves cultured in sugar solution (18), serves to strengthen this analogy.

Finally, it seems worth while to draw attention to the importance of the translocation of metabolites into the tissue under attack by an obligate parasite from surrounding healthy tissue, and to the possibility which exists for the movement of metabolites, etc. out of the infections into the surrounding tissue. Transport into infection zones provides metabolites at a time when photosynthesis is depressed and respiration is much increased. The relative rates of transport into and utilization of metabolites within the infection zone may well be an important factor in determining both the degree of development of the parasite and the reaction of the host.

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CANADIAN SPECIES OF SYNCHYTRIUM[1]

By John S. Karling[2]

Abstract

Twenty collections of *Synchytrium* from various parts of Canada were studied, and among these were *S. perforatum*, *S. athyrii*, *S. rubrocinctum*, *S. cupulatum*, and *S. aureum*. This is the first report of *S. athyrii*, *S. rubrocinctum*, and *S. cupulatum* from America. In addition to these five species, 13 short-cycled ones are described. These cannot be identified with certainty at present from the material at hand, and are not given specific names. Most of them occur on new hosts in new localities, and the collection of them greatly extends the known geographical and host range of *Synchytrium*.

Synchytrium is a large genus of chytridiaceous parasites which is widely distributed in the United States and elsewhere, but as far as I am aware only four species have been reported from Canada. Apparently, this is not due to a scarcity of species there, but because these fungi have not received much attention in the past from Canadian mycologists. At least, this is indicated by the large number of specimens collected in recent years by Dr. D. B. O. Savile and his associates in the Department of Agriculture, Ottawa. Many of these species occur on unrecorded hosts in British Columbia and the Northwest Territories, and the collection of them greatly extends the host range and geographical distribution of *Synchytrium*. The present contribution concerns the morphology, host reaction, and hosts of these Canadian species. Most of them are short-cycled, apparently develop only resting spores, and appear to be members of the subgenus *Pycnochytrium*. Because they are incompletely known, many of them cannot be identified with certainty, and for this reason are not given specific names. Nevertheless, such species are described with the purpose of calling attention to their occurrence and providing other collectors with data for comparisons.

The herbarium specimens were first studied in the dried condition after which infected parts of the hosts were thoroughly soaked in water, fixed in a vacuum to remove air, embedded in paraffin, sectioned, and stained. The host tissue in such sections was badly shrunken, but the fungi were sufficiently well preserved to show their essential characteristics. However, they also were slightly shrunken, and as a result the dimensions given below are probably somewhat smaller than they would be in living material. The specimens of these fungi are in the Mycological Herbarium, Department of Agriculture, Ottawa, Canada, and the numbers cited below refer to the specimen numbers in the Canadian herbarium.

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Synchytrium perforatum Ellis. *In* Karling, Bull. Torrey Botan. Club, 82 : 219. 1955

This species was collected by J. A. Calder, D. B. O. Savile, and J. M. Ferguson, 22 July, 1954, at Red Rose Tungsten Mine, Rocher Deboulé Mts. near Hazelton, B.C., on *Valeriana sitchensis* at an altitude of 4700 ft. The Canadian specimens (DAOM 45856) are identical to *S. perforatum* collected by Suksdorf on the same host, and *Valerianella congesta* in Oregon and Washington, and to the one I (3) described on *Valerianella stenocarpa* from College Station, Texas. Savile's collection also includes specimens of this species on *Valeriana sitchensis* which Calder collected on Mt. Marathon, Kenai Peninsula, Alaska. Recently I found it on *Valerianella macrocera* in the herbarium of the University of California, and during the summer of 1955 Dr. G. B. Cummins collected it on *Valeriana sitchensis* in Montana. Accordingly, so far as it is known this species ranges from Texas to Alaska and occurs at almost sea level to 4700 ft. altitude. In a further study of *S. perforatum* on *Valerianella macrocera* and *V. congesta*, I found that the number of perforations in the infected cell wall may be up to 400 or more, and that radial striations similar in appearance to plasmadesmata connect the perforations.

Synchytrium athyrii Lagerheim. Scheda zu Vestergren's micro. rar. sel. 1909, 1893

This species was collected on *Cystopteris fragilis* by Calder, Savile, and Ferguson, 7 July, 1954, on a mountain slope north of Azouzetta L., Pine Pass, B.C. The characteristics of the Canadian specimens are as follows: Resting spores solitary or up to three in a cell, usually spherical, 88–108 μ, with a brown, smooth wall, 2.5–3 μ thick, and light-brown granular content; enveloped by a small amount of brown residue; germination unknown. Simply monogallic, resting-spore galls separate and scattered or crowded on the fronds and rachis, obpyriform or broadly clavate, 126–150 $\times$ 162–170 μ, or ovoid, 120–180 $\times$ 174–204 μ, with a hyaline wall, 2.8–4 μ thick, which becomes tan to light-brown with age; galls frequently invaginated at apex when dry and becoming cupulate.

The resting spores of the Canadian species are about equal in size to those of Lagerheim's fungus, but the galls which the former induces are somewhat smaller. Nevertheless, I believe the two fungi are identical. In the event this proves to be so, this will be the first report of *S. athyrii* outside of Norway. I made several attempts to germinate the resting spores of the Canadian fungus, but these were unsuccessful.

Synchytrium cupulatum Thomas. Botan. Centr. 29 : 42. 1887

This species was collected by Savile and Watts, 15 August, 1950, on *Dryas integrifolia* at Chesterfield Inlet, Dist. of Keewatin, N.W.T. and Belcher Island, Hudson Bay, by Mrs. R. Jeffrey. Its characteristics are as follows: Resting spores usually solitary, spherical, 70–117 μ, or ovoid, 94–120 $\times$

102–138 μ, with a smooth, amber wall, 2.8–3.5 μ thick, and coarsely granular hyaline content; enveloped by a small amount of crusty, brown residue; germination unknown. Simply monogallic, resting-spore galls usually aggregated, almost spherical, 120–174 μ, or broadly obpyriform, 120–218 μ in broadest diameter, by 162–230 μ high with a hyaline wall, 2.5–3 μ thick, and containing a dark-lavender to lavender-violet pigment; becoming cup-shaped after drying out.

Dryas integrifolia is a new host for this species. When its young leaves are heavily infected on the underside they become thickened and curl upward. The resting spores and galls in the Canadian material are slightly smaller than those of European specimens of *S. cupulatum*, but I believe the two fungi are identical. This is the first record of *S. cupulatum* in North America, although Rostrup (10) and Larsen (8) reported it in Iceland.

Synchytrium rubrocinctum (?) Magnus. Hedwigia, 13 : 107. 1874

This species was collected by Savile and Watts, 27 July, 1950, on *Saxifraga cernua* at Chesterfield Inlet, Dist. of Keewatin, N.W.T. It has the following characteristics: Resting spores solitary, partly filling host cell, spherical, 60–90 μ, or ovoid, 34–93 × 38–102 μ, with a smooth hyaline wall, 2–3.5 μ thick, and hyaline content; enveloping residue sparse or abundant; germination unknown. Simply monogallic, resting-spore galls separate and scattered or crowded at margin of leaves, filled with a lavender-red pigment which gives the galls a dark-violet color when they dry out, broadly obpyriform to broadly clavate, 108–240 μ high by 105–156 μ in broadest diameter, or subspherical, 95–170 μ, invaginating at the sides and apex to become distinctly polyhedral after drying out.

This fungus is identified tentatively as *S. rubrocinctum* which Magnus, Tolf (14), and Vill (15) reported on *Saxifraga granulata* in Germany and Sweden. I have compared it carefully with material of *S. rubrocinctum* collected by Vestergren in Sweden in 1897, and both collections are very similar. Magnus reported that the wall of the resting spore is unevenly rough, but I found only smooth walls in Canadian and Vestergren's fungi. Except for its hyaline wall this fungus is also fairly similar to *S. cupulatum* which was described above. Both species were found in the same locality, and it is possible that with further study they may prove to be identical.

Synchytrium aureum (?) Schroeter. Hedwigia, 9 : 4. 1870

This species was collected by Calder and Savile, 2 August, 1953, on *Parnassia parviflora*, one-half mile west of Athalmer, near Windermere, B.C., and identified by Savile as *S. aureum*. In the material which I studied the galls are comparatively sparse on the leaves and petioles but fairly abundant on the lower part of the stem. In addition this fungus has the following characteristics: Resting spores usually solitary, filling host cell almost completely or only partly, spherical, 150–195 μ, or ovoid, 130–145 × 150–165 μ, with an amber, smooth wall, 3.5 μ thick, and brilliantly yellow to yellowish-amber content; enveloping residue sparse; germination unknown.

Compositely monogallic, resting-spore galls separate and scattered, but frequently confluent on stem, protruding conspicuously, almost hemispherical, 280–320 μ high by 420–460 μ broad at the base, with a shallow apical crater; sheath 3–5 cells thick; sheath cells greatly enlarged with walls up to 6.2 μ thick.

Whether or not this is *S. aureum* remains to be proved. Its spores fall within the size range of those reported for *S. aureum* but the induced galls are much larger. *Synchytrium aureum* has been reported by Juel (2) on *Parnassia palustris* and several other species of the Saxifragaceae, but a description of the fungi on these hosts was not given. Therefore, it is difficult to make comparisons. It may be noted here that R. F. Daubenmire, 11 August, 1947, collected *Parnassia fimbriata* at Granite Creek, Roosevelt Grove, Wash., on which C. G. Shaw later found a species of *Synchytrium*. It is likewise compositely monogallic, and its resting spores are very similar in size and shape to those of the Canadian fungus. However, the galls induced by the Washington fungus are much larger and have an apical rosette of elongate to clavate sheath cells.

Synchytrium spp. on *Collinsia parviflora*, *Delphinium menzeisii*, and *Sedum douglasii*

These new hosts of *Synchytrium* were collected by Calder and Savile, 20 June, 1953, in close proximity at an altitude of 4000 ft. on a mountain slope five and one-half miles southwest of Rossland, B.C., and with one exception the fungi on them may prove to be identical. For the purpose of comparing them carefully a description is given for each fungus. The species on *Collinsia parviflora* has the following characteristics : Resting spores solitary, spherical, 104–156 μ, or ovoid, 120–140 $\times$ 135–156 μ, with an amber-brown, smooth wall, 3–4 μ thick, and yellow content; enveloping residue sparse or lacking; germination unknown. Compositely monogallic, resting-spore galls on both surfaces of leaves and sepals, separate and scattered or aggregated and confluent, dark lavender-red to violet, mound-shaped or hemispherical, 312–364 μ high by 480–540 μ broad; sheath 3–5 cells thick. The galls induced by this species become quite soft when soaked in water, lose their color, and may be dissected readily to remove the brilliantly yellow resting spores.

The species on *Delphinium menzeisii* is scarce and consists only of a few galls and spores on the leaves and stem. Therefore, the present description may not be representative. The resting spores are usually solitary, spherical, 95–140 μ, with a smooth wall, 3–4 μ thick, and yellow content, and are enveloped by a sparse amount of residue. The method of germination is unknown. In relation to its host this fungus is compositely monogallic with scattered mound-shaped or hemispherical galls, 280–320 μ high by 400–500 μ broad, whose sheaths are 2–5 cells thick. As far as it is known this species is slightly smaller but otherwise very similar to the one on *Collinsia parviflora*.

Apparently, two species are present on *Sedum douglasii*. The more abundant one is characterized by: resting spore usually solitary, spherical, 120–170 μ, or ovoid, 115–132 × 128–140 μ, with an almost hyaline to light-amber wall, 3.5–4 μ thick, and bright yellow content; enveloping residue sparse; germination unknown. Compositely monogallic, resting-spore galls separate and scattered or crowded and confluent on both surfaces of leaves, greenish-yellow, hemispherical or mound-shaped, 216–276 μ high by 266–420 μ broad; sheath 3–5 cells thick, sheath cells greatly enlarged and thin-walled. The galls of this species may be very abundant and cause crinkling, distortion, and stunting of young leaves. As in the species on *Collinsia* the galls become soft upon soaking in water so that the resting spores may be dissected out quite readily. This fungus is quite similar to the two described above, and it is quite possible that they are identical. In this connection it may be noted that Shaw and Yerkes, 8 June, 1949, collected a species on *Sedum douglasii* at Teals Springs, Garfield Co., Wash., which I (4) described previously. Its resting spores and galls are similar in size and structure to those of the Canadian fungus, and it is quite likely that the two fungi are identical.

The second species on *Sedum douglasii* in the Canadian collection is very scarce on the leaves and induces simple scattered galls which consist of an enlarged and protruding epidermal cell. However, the galls on the dried *Sedum* leaves are so shrunken that it is difficult to determine their exact shape and size. From the limited information at hand the fungus may be described as follows: Resting spores usually solitary, sometimes up to three in a cell, filling host cell almost completely or partly, predominantly oval, 32–52 × 43–66 μ, with a reddish-brown, smooth wall, 4–6 μ thick; enveloping residue fairly abundant, compact, and dense; germination unknown. Simply monogallic, resting-spore galls mound-shaped or broadly obpyriform, 60–78 μ high by 75–90 μ in broadest diameter, wall 3–5 μ thick and reddish-brown.

From the limited material at hand it is difficult to determine the identity of this species. It may prove to be identical or related to one of the many simply monogallic species of the subgenus *Pycnochytrium*.

Synchytrium sp. on *Viola pennsylvanica*

Resting spores solitary, partly filling host cell, spherical, 54–82 μ, or ovoid, 58–65 × 72–90 μ, with a smooth, amber-brown wall, 3.5–4.2 μ thick, and yellowish content; enveloping reddish-brown residue filling remainder of host cell and extending up into neck of gall; germination unknown. Compositely monogallic, resting-spore galls usually crowded and confluent and forming raised plaques or ridges, or separate and scattered, protruding conspicuously, and largely superficial, subspherical with a slightly constricted base, or almost hemispherical, 192–268 μ high by 170–270 μ broad, with a shallow apical crater; sheath 3–5 cells thick.

On leaves and petioles of *Viola pennsylvanica*, Harrow, Ontario (Leg. I. L. Conners and J. A. Parmelee, 23 May, 1950; DAOM 46089).

This may be *S. aureum* which has been reported on 15 species of *Viola*. However, it has not been described on any of these hosts, which makes com-

parison with the present species difficult. The resting spores are generally smaller than those reported for *S. aureum* on other hosts, but they are enveloped by a dense layer of residue which extends up into the apex of the infected cell as was originally illustrated by Schroeter (12) in *S. aureum*. *Synchytrium globosum* also has been reported on nine species of *Viola*, but in this fungus the content of the resting spore is reported to be hyaline instead of orange to yellow.

Synchytrium sp. on *Epilobium palustre*

Resting spores solitary, partly filling host cell, spherical, 80–120 μ, or ovoid, 40–96 × 60–134 μ, with a smooth wall, 2.4–4 μ thick, and golden-brown content; enveloping residue abundant, up to 18 μ thick, and usually filling remainder of host cell; germination unknown. Compositely monogallic, resting-spore galls separate and scattered, or crowded and confluent, embedded largely in host tissue and not protruding conspicuously, comparatively small, mound-shaped, 90–156 μ high by 120–180 μ broad; sheath 1–3 cells thick, sheath cells greatly enlarged.

On lower leaves and lower part of stems of *Epilobium palustre*, Brabant Island, Great Slave Lake, Dist. of Mackenzie, N.W.T. (Leg. W. H. Lewis, 31 July, 1951; DAOM 45840).

As I pointed out previously (5) in a brief description this may be *S. aureum*, or at least identical to the species which Schroeter (13) reported but did not describe as *S. aureum* on *E. palustre* and other species of *Epilobium* in Germany.

Synchytrium sp. on *Lupinus rivularis*

Resting spores solitary or up to three in a host cell and partly filling it, oval 96–130 × 135–160 μ, or spherical, 90–134 μ, with a smooth, amber-brown wall, 3–3.8 μ thick, and densely alveolar yellow content; enveloping residue fairly abundant, or sparse or lacking; germination unknown. Compositely monogallic, resting-spore galls on both surfaces of leaf, greenish with yellow spore showing through the sheath, scattered and separate or sometimes confluent, galls on leaf protruding conspicuously, mound-shaped or hemispherical, 270–360 μ × 320–460 μ broad with a narrow, up to 48 μ diam. apical crater; sheath 3–5 cells.

On leaves and petioles of *Lupinus rivularis*, 6000 ft. alt., east slope of Frosty Mt., Manning Prov., B.C. (Leg. Calder, Savile, and Ferguson, 9 September, 1954; DAOM 45868).

The species frequently infects the trichomes and these are stimulated to divide and develop into somewhat cone-shaped galls which may be up to 748 μ high by 404 μ broad. The resting spores in this material appear to be immature in that their content is densely alveolar and does not include the commonly present granules and globules. This is the first species of *Synchytrium* to be reported on *Lupinus*, but according to herbarium specimens at the State College of Washington, C. G. Shaw, 20 July, 1950, collected a fairly similar fungus on *Lupinus leucopsis* in Chelan Co., Wash.

Synchytrium sp. on *Primula mistassinica*

Resting spores usually solitary, sometimes up to three in a cell, usually filling host fully, sometimes only partly, spherical, 96–132, or ovoid, 70–120 × 90–126 μ, small ones 38–50 × 52–65 μ, with a smooth wall, 3.6–4.2 μ thick, and lemon-yellow content; enveloping residue reddish-brown and compact, sparse or fairly abundant; germination unknown. Simply monogallic, resting-spore galls sometimes consisting of an enlarged, largely embedded oval, oblong or spherical epidermal cell, 80–130 μ diameter; or compositely monogallic, galls lowly mound-shaped or subhemispherical and protruding only slightly with a broad crater, 120–132 μ high by 144–192 μ broad; sheath 1–3 cells, consisting usually of outwardly elongate epidermal cells with thickened walls.

On leaves of *Primula mistassinica*, Kinbasket L., 60 miles northwest of Golden, B.C. (Leg. Calder and Savile, 16 August, 1953; DAOM 45878).

The galls induced by this species vary considerably from single, enlarged, and mostly embedded epidermal cells to lowly mound-shaped to almost hemispherical protuberances with sheath cells extending part to almost all the way around the infected cell. However, the latter is usually largely exposed. The lateral sheath cells in composite galls are outwardly elongate and enlarged epidermal cells with fairly thick walls. Usually, the epidermal cells between fairly close galls are enlarged also. Many of these galls resemble Rytz's (11) drawing of those caused by *S. aureum* on *Saxifraga aizoides*, *Hutchinsia alpina*, and *Viola biflora*. The resting spores of the Canadian species are generally smaller than those of *S. aureum*, but it is possible that the two fungi are identical. *Synchytrium aureum* was reported on *Primula officinalis* by Schroeter (13) in Germany, but he did not describe it on this host. I have made repeated attempts to germinate the resting spores from the Canadian host without being successful.

Synchytrium sp. on *Pedicularis racemosa*

Resting spores usually solitary, sometimes up to three in host cell; filling it almost completely, ovoid, 60–90 × 72–100 μ, or subspherical to spherical, 60–104 μ, with a smooth wall, 4–6 μ thick, and yellowish-orange content; enveloping residue usually sparse, germination unknown. Compositely monogallic, resting-spore galls equally abundant on both surfaces of leaves, separate and scattered, or frequently crowded and confluent, frequently lavender-violet to dark-violet, usually protruding conspicuously, volcano-like or almost hemispherical with a fairly broad crater, 86–216 μ high by 98–220 μ broad at base; sheath 1–3 cells thick, walls of sheath and infected cells greatly thickened.

On leaves and petioles of *Pedicularis racemosa*, 6000 ft. alt., east slope of Frosty Mt., Manning, Prov. Pk., B.C. (Leg. Calder, Savile, and Ferguson, 9 Sept., 1954; DAOM 45871).

This species is very abundant and causes marked thickening, up to 300 μ, of the leaves where the galls are confluent. It may prove to be identical to *S. aureum* which Schroeter (13), Juel (2), and Davis (1) reported on

Pedicularis silvatica, *P. palustris*, and *P. canadensis*, respectively, in Germany, Sweden, and Wisconsin, U.S.A. These collectors did not describe their fungi, and it is impossible to compare them with the Canadian species.

Synchytrium sp. on *Potentilla simplex*

Resting spores usually solitary, rarely two in a cell, filling about one-half of host cell, spherical, 110–128 μ, or ovoid, 90–98 $\times$ 115–130 μ, with a smooth wall, 3.8–5 μ thick, and light-yellow content; enveloping reddish-brown residue compact, homogeneous and filling remainder of host cell, or relatively sparse; germination unknown. Compositely monogallic, resting-spore galls separate and scattered, or frequently crowded and confluent, dark lavender-violet, almost hemispherical or mound-shaped, with broad or narrow crater at apex, 216–270 μ high by 220–360 μ broad, base embedded in leaf, or frequently bulging out on opposite side of leaf; wall in infected cell greatly thickened; sheath 1–4 cells thick, sheath cells enlarged.

On leaves and petioles of *Potentilla simplex*, Essex Co., Ont., Can. (Leg. J. A. Parmelee, 9 June, 1950; DAOM 46090).

In most of the galls the resting spores are enveloped by a layer of globular or irregular residue which makes the spores appear as if they are coarsely verrucose, but when this layer is stripped off the spore wall is smooth. Adjacent to and outside of this verrucose layer the residue is usually reticulate and cytoplasm-like with a few dispersed globules, and immediately inside of the wall of the infected cell occurs another verrucose layer with the knobs projecting inward. These layers give the thick-walled infected cell with the spore a characteristic appearance in sectioned and stained preparations. In other galls the residue is dense, compact, homogeneous, and fills the remainder of the host cell.

This is the first compositely monogallic species to be reported on *Potentilla simplex*, but *S. globosum* and *S. aureum* have been recorded on *P. reptans* by Schroeter (13), Vill (15), and Rytz (11) in Germany. The content of resting spores of *S. globosum*, however, is hyaline while that of the present Canadian is light-yellow to yellow. The galls which the latter induces resemble those of *S. aureum* figured by Rytz (11, p. 7), and its spores fall within the size ranges reported for that species. It is quite possible that the two fungi will prove to be identical.

Synchytrium sp. on *Hydrophyllum capitatum*

Resting spores usually solitary, sometimes two in a cell, filling only a small part of greatly enlarged host cell, spherical, 90–114 μ, or ovoid, 72–90 $\times$ 120–144 μ, with a smooth reddish-brown wall, 3–3.6 μ thick, and hyaline content; enveloped by a layer of reddish residue; germination unknown. Compositely monogallic, resting spore galls abundant on lower surface of leaf, usually crowded and often confluent, whitish with a dark-purple basal zone at first but becoming reddish-brown, hemispherical or mound-shaped with a broad shallow apical depression, 168–228 μ high by 290–336 μ broad, sheath 1–3 cells thick.

On leaves of *Hydrophyllum capitatum*, one mile northwest of Mt. Apex, 6750 ft. alt., near Penticton, B.C. (Leg. Savile and Calder, 19 July, 1953; DAOM 45877).

The host cell becomes unusually enlarged, obpyriform to oval in shape and may be 180–224 × 230–268 μ in diameter with a hyaline wall up to 8 μ thick. The resting spore fills only a part of this cell, and in the immature stages the remainder is filled with hyaline, foamy, or reticulate cytoplasm-like residue. This imparts a white color to the cell and gall, except for the dark-purple basal layer of sheath cells. The galls are thus very striking in appearance at this stage. Later, the residue becomes faintly purplish and finally condenses somewhat to become reddish-brown and resin-like. The unicellular trichomes may be infected when quite young and swell up into large obpyriform cells with a basal fringe of sheath cells.

This species does not appear to be *S. aureum* because of the hyaline content of the resting spores, and for this reason I am including it temporarily in *Leucochytrium*. So far only two other species of *Synchytrium* have been reported (Karling (6, 7)) on members of the *Hydrophyllaceae*, *S. namae* on *Nama hispidum*, and *Synchytrium* sp. on *Hesperochiron pumulus*. *Synchytrium namae* is a simply monogallic species with minute resting spores and thus quite different from the present species. The fungus on *Hesperochiron* is compositely monogallic, but its resting spores are usually much larger than those of the one on *Hydrophyllum*. I have made several attempts to germinate the resting spores of the latter species without being successful.

Synchytrium sp. on *Mentha arvensis*

Resting spores solitary, partly filling and lying in base of greatly enlarged flagon-shaped, thick-walled host cell, spherical, 144–215 μ, with an amber-brown, smooth wall, 3–3.9 μ thick, and brilliantly yellow to yellowish-orange content; enveloped by a fair amount of dark amber-brown residue; germination unknown. Compositely monogallic, resting-spore galls abundant on both surfaces of leaves and on stems, scattered or crowded and sometimes confluent, reddish-brown to dark-lavender when mature, very large and protruding conspicuously, subspherical to mound-shaped or almost hemispherical with a slightly constricted base and a broad apical depression, usually flared out near the apex, 240–468 μ high by 336–610 μ broad; sheath 3–7 cells thick, sheath cells elongate and greatly enlarged.

On leaves and stems of *Mentha arvensis*, southeast shore of Mara Lake between Enderby and Sicamous, B.C. (Leg. Calder and Savile, 14 July, 1953; DAOM 45874).

This species induces unusually large galls with a greatly enlarged host cell which is usually flagon-shaped and whose wall may be up to 9 μ thick. This may possibly prove to be *S. aureum* which has been reported on 13 genera and 15 species of the Labiatae, including *Mentha aquatica* (Schroeter (13)). However, *S. aureum* on such hosts has not been described and it is difficult to

compare it with the Canadian species. The latter is considerably larger than *S. aureum* as described and figured by Schroeter (13) and Rytz (11) and may prove to be a different species. So far I have not succeeded in germinating the resting spores.

Synchytrium sp. on *Gentiana sceptrum*

Resting spores solitary or sometimes up to three in a cell, spherical, 186–220 μ, or sometimes oval, 175–190 × 200–210 μ, with an amber wall, 3–4 μ thick, and brilliantly lemon-yellow content; enveloped by a sparse amount of residue; germination unknown. Compositely monogallic, resting-spore galls separate and scattered, or crowded and confluent, greenish with yellow spore showing through sheath, old galls sometimes lavender-red, separate galls protruding conspicuously, dome-shaped or hemispherical with base embedded in host tissue, 200–420 μ high by 380–500 μ broad; sheath 3–5 cells thick.

On both surfaces of leaves of *Gentiana sceptrum*, 11 miles southeast of Prince Rupert, B.C., Can. (Leg. Calder, Savile, and Ferguson, 25 Aug., 1954).

The separate and scattered galls induced by this species may be quite large, but when they occur close together they are smaller. The resting spores of this fungus also are comparatively large.

In connection with this fungus it may be noted C. G. Shaw also collected a *Synchytrium* species on *Gentiana* in Washington which has the following characteristics: Resting spores usually solitary, sometimes up to three in a cell, and filling it only partly, subspherical, 90–144 μ, or ovoid, 80–100 × 120–130 μ, with a smooth wall, 3.8–4 μ thick, and faintly yellow to hyaline content; enveloping residue sparse or lacking; germination unknown. Compositely monogallic, resting-spore galls separate and scattered, or crowded and confluent, greenish with hyaline to faintly yellow spores showing through sheath, usually dome-shaped with a shallow apical crater 168–240 μ high by 312–408 μ broad, sometimes protruding almost equally on both sides of leaf, or only on one side; sheath 4–6 cells thick, sheath cells greatly enlarged.

On leaves of *Gentiana oregana*, Neal, Garfield Co., Wash., U.S.A. (Leg. C. G. Shaw, 21 May, 1951; spec. no. 31286, State Coll. Wash.).

Except for its smaller and almost hyaline spores this species is similar to the one on *G. sceptrum* from Canada. The Washington material appears to be immature, and it is possible that the differences noted in the resting spores may be due to this condition. So far only one other species of *Synchytrium* has been reported on members of the Gentianaceae: *S. aureum* on *G. campestre* (2), *G. pseudoaquatica* (9), and *Halenia deflexa* (1). None of these investigators, however, described their fungus, and it is impossible to compare it with the Canadian and Washington species.

In addition to the species described as well as *S. aecidioides*, *S. aureum*, and *S. vaccinii* which were reported previously, three other species have been collected in Canada. These include *S. myosotidis* on *Plagiobotrys*

hirtus (Leg. J. R. Anderson, 10 May, 1896, near Esquimalt, Vancouver Island, B.C.; spec. in herb. State Coll. Wash.); *Synchytrium* sp. on *Phlox gracilis* (Leg. W. B. Anderson, 14 May, 1918, Darcy, B.C.; spec. in herb. State Coll. Wash.) which I described briefly (6); and a species on *Claytonia virginica* (Leg. I. L. Conners and J. A. Parmalee, 26 May, 1950, Essex Co., Ont.) which will be described shortly by Dr. D. B. O. Savile.

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NOTE

A SIMPLE AND INEXPENSIVE METHOD OF SHIPPING CULTURES OF FUNGI BY AIR MAIL[1]

By W. L. Gordon[2]

Since World War II it has been common practice to ship viable fungus cultures long distances by air mail in order to reduce the time in transit as much as possible. The usual method employed in the preparation of cultures for shipment is to grow the fungi on agar slants in test tubes. Each test tube is then wrapped in cotton wool or some other satisfactory insulating material and packed securely in a strong carton. Some investigators prefer to enclose each test-tube culture in a separate wooden container which has been designed for shipping purposes, or in separate compartments of a larger one of the same type holding two or more test tubes. These containers are then usually wrapped together with strong paper before mailing. Because of the weight of the agar, test tubes, containers, and the other wrapping materials, the air-mail postage to other continents may be a considerable amount. There is also the danger that the test tubes might break during transit and this damage would render more difficult the recovery of the cultures.

As a simple and more economical method of shipping fungus cultures by air mail is desirable, the following method has been developed and has been found to be particularly advantageous from the standpoints of (1) a reduction in the time and labor involved in the preparation of the cultures for shipment, (2) a significant reduction in the air-mail postage, and (3) freedom from damaged containers during transit.

The method, which consists of two parts, is as follows:

(1) With a sterile scalpel or needle transfer from a Petri-plate or test-tube culture a piece of agar and fungus about 1 cm. square and 5 mm. deep and insert the material carefully into a small cellophane envelope. Transfers should be made from relatively young cultures, that is, from cultures one to two weeks old, rather than from old cultures of doubtful vitality. After insertion of the piece of agar and fungus into the envelope the open side is folded over once or more and sealed with cellulose tape. As the gum on most types of labels does not adhere well to cellophane, the culture identification label is attached to the envelope with the cellulose tape at the time it is sealed. It has not been found necessary to sterilize new cellophane envelopes before use but they can be sterilized by placing them in a liter jar containing 1 cc. of propyline oxide for 24 hr. The lid of the jar should be sealed. At least 10 of these cellophane-envelope cultures can be enclosed in a letter in an air-mail envelope of ordinary size.

[1]*Contribution No. 1512 from the Botany and Plant Pathology Division.*
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(2) The procedure recommended for recovery of a fungus culture from the cellophane envelope is to slit open the envelope, scrape the agar and fungus from it with a sterile scalpel, and then place them in a test tube containing about 10 cc. of acidified, sterile water. The test tube is then shaken vigorously to break up clumps of conidia, mycelium, and agar. This suspension is then poured immediately over the surface of one or more water-agar plates. The excess liquid is decanted off slowly and the plates are then incubated for 16–24 hr. at room temperature. After the period of incubation the surface of the plates is examined under a dissecting microscope (about 35×) for germinating conidia and new hyphae. Conidia or hyphal tips, and a small bit of adhering agar, are picked up singly with a sterile needle, and transferred to separate tubes of potato-sucrose agar. This method of recovering the fungus, compared with the usual mass transfer procedure, eliminates any difficulties that arise if contaminants should be present.

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